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(21) International Application Number: PCT/US92/10983 (22) International Filing Date: 17 December 1992 (17.12.92) (30) Priority data: 07/810,279 17 December 1991 (17.12.91) US 07/853,408 18 March 1992 (18.03.92) US 07/904,068 23 June 1992 (23.06.92) US (60) Parent Application or Grant (63) Related by Continuation US 07/904,068 (CIP) Filed on 23 June 1992 (23.06.92) (71) Applicant (for all designated States except US): GEN- PHARM INTERNATIONAL, INC. [US/US]; 2375 Garcia Avenue, Mountain View, CA 94043 (US).		(72) Inventors; and (75) Inventors/Applicants (for US only) : LONBERG, Nils [US/ US]; 156 Carmel, San Francisco, CA 94117 (US). KAY, Robert, M. [US/US]; 2127 Broadway #5, San Francisco, CA 94115 (US). (74) Agents: SMITH, William, M. et al.; Townsend and Town- send, One Market Plaza, 2000 Steuart Tower, San Fran- cisco, CA 94105 (US). (81) Designated States: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, RO, RU, SD, SE, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: TRANSGENIC NON-HUMAN ANIMALS CAPABLE OF PRODUCING HETEROLOGOUS ANTIBODIES (57) Abstract <p>The invention relates to transgenic non-human animals capable of producing heterologous antibodies and transgenic non-human animals having inactivated endogenous immunoglobulin genes. In one aspect of the invention, endogenous immunoglobulin genes are suppressed by antisense polynucleotides and/or by antiserum directed against endogenous immunoglobulins. Heterologous antibodies are encoded by immunoglobulin genes not normally found in the genome of that species of non-human animal. In one aspect of the invention, one or more transgenes containing sequences of unrearranged heterologous human immunoglobulin heavy chains are introduced into a non-human animal thereby forming a transgenic animal capable of functionally rearranging transgenic immunoglobulin sequences and producing a repertoire of antibodies of various isotypes encoded by human immunoglobulin genes. Such heterologous human antibodies are produced in B-cells which are thereafter immortalized, e.g., by fusing with an immortalizing cell line such as a myeloma or by manipulating such B-cells by other techniques to perpetuate a cell line capable of producing a monoclonal heterologous antibody. The invention also relates to heavy and light chain immunoglobulin transgenes for making such transgenic non-human animals as well as methods and vectors for disrupting endogenous immunoglobulin loci in the transgenic animal.</p>		

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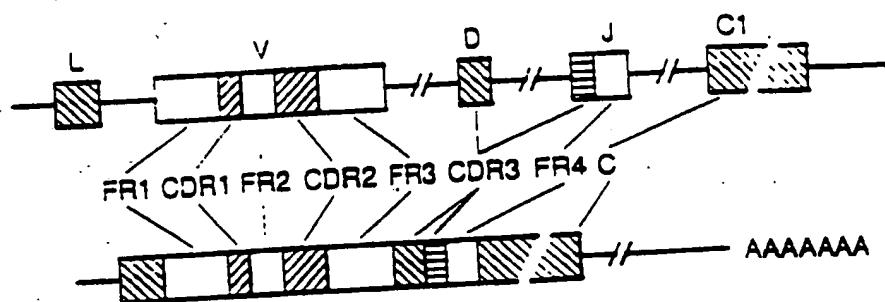


FIG. 1

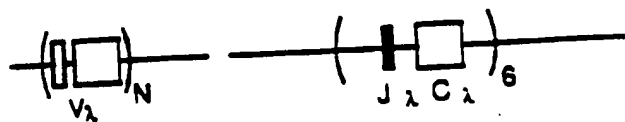


FIG. 2

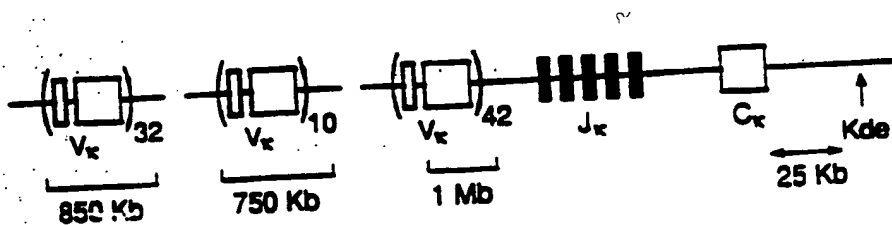


FIG. 3

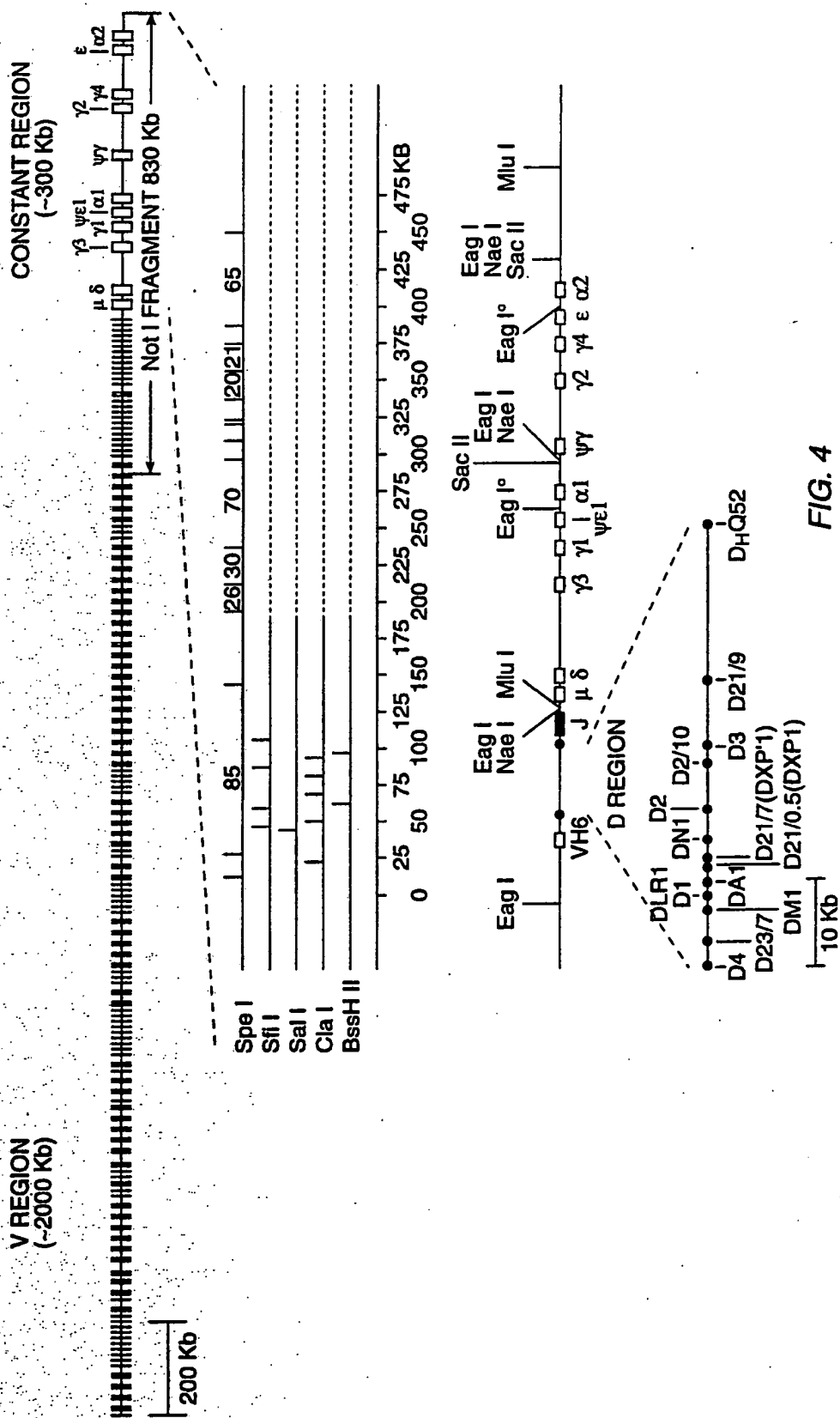


FIG. 4

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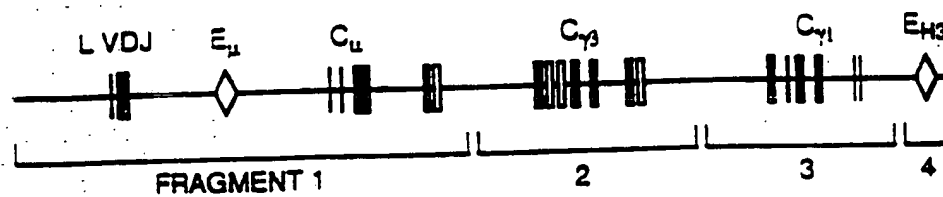


FIGURE 5

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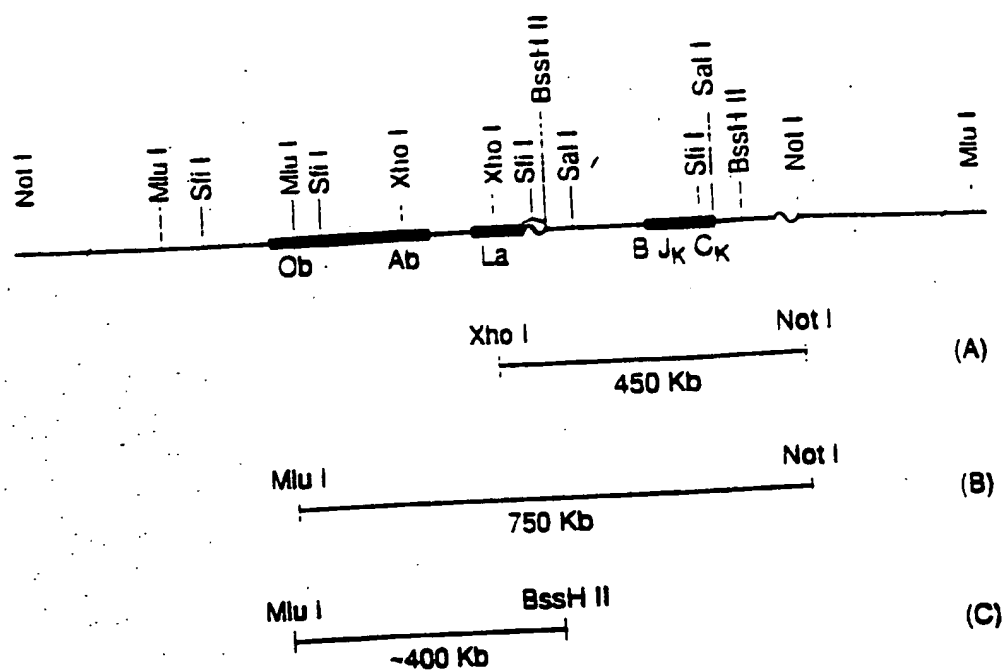


FIGURE 6

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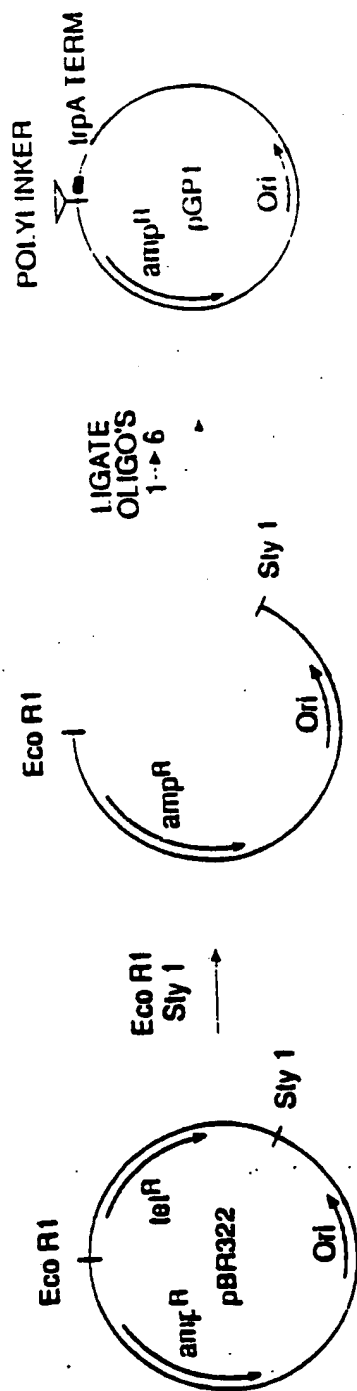


FIGURE 7

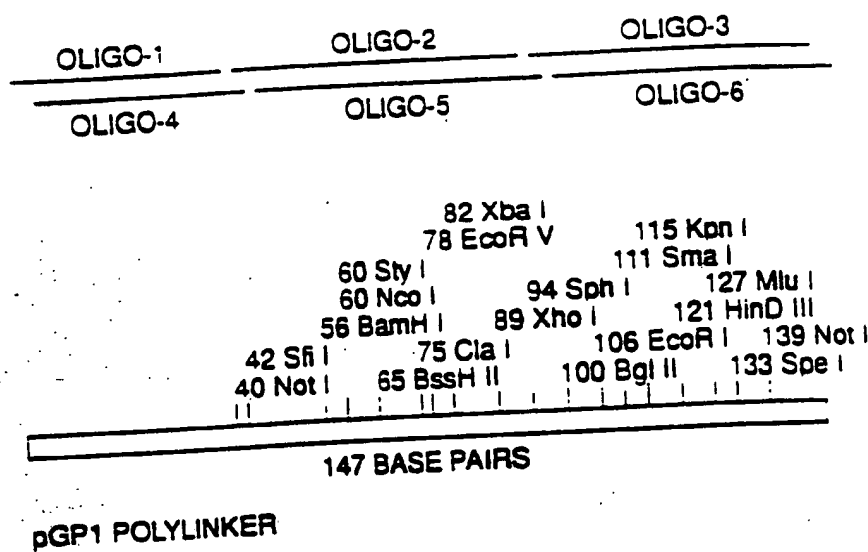


FIGURE 8

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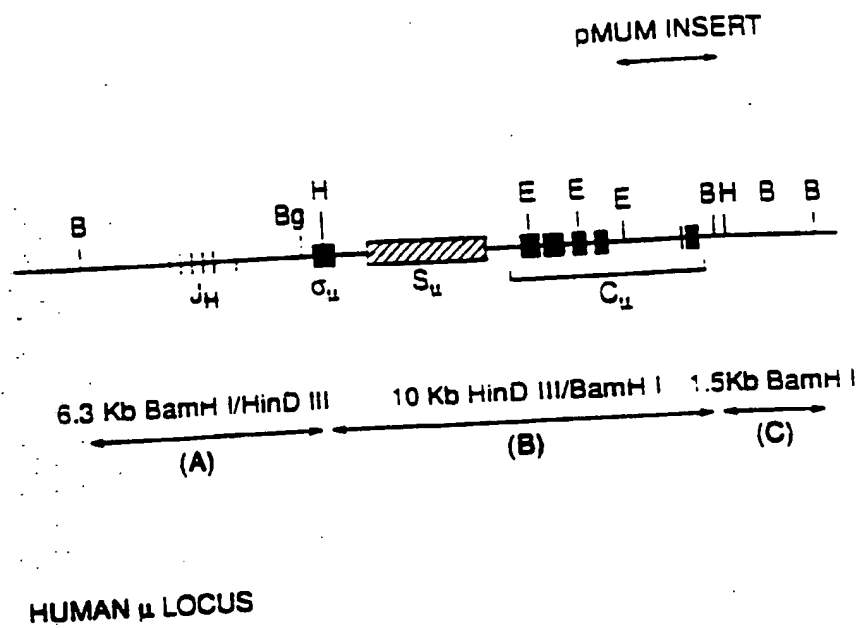


FIGURE 9

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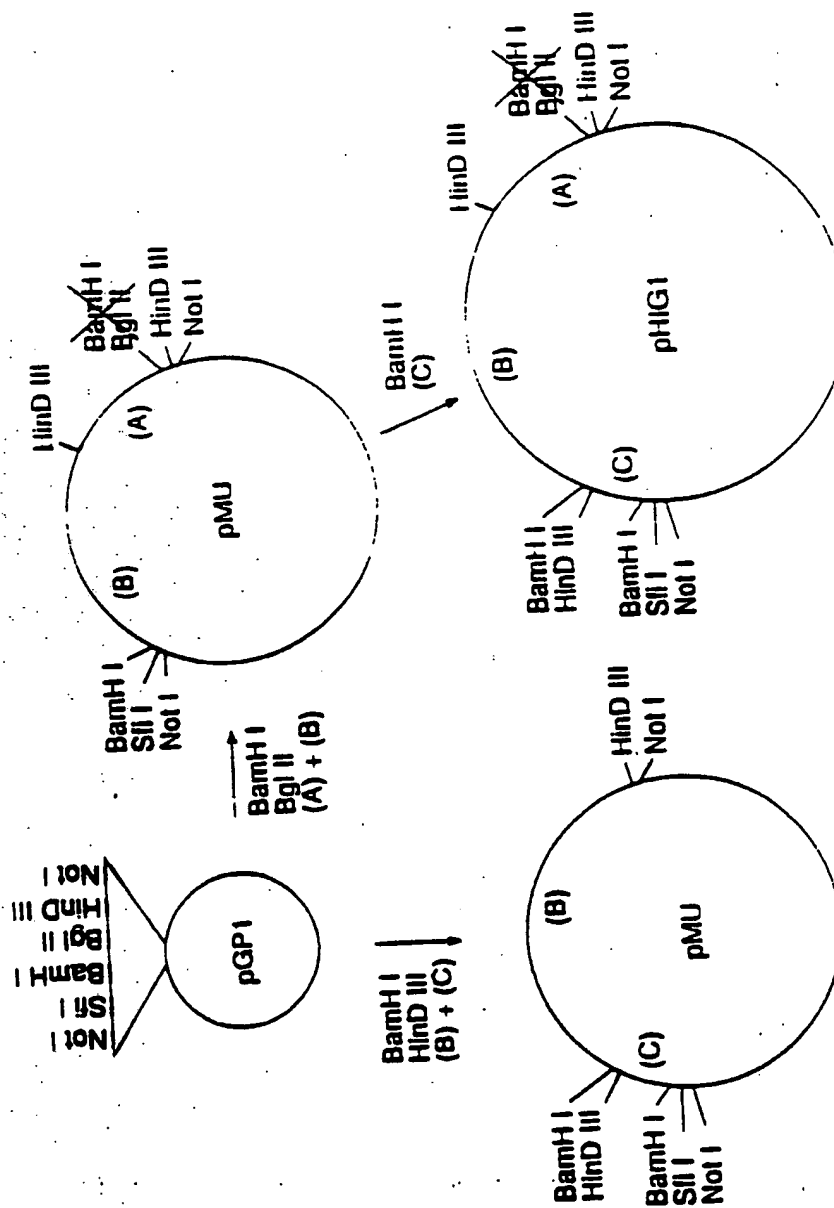


FIGURE 10

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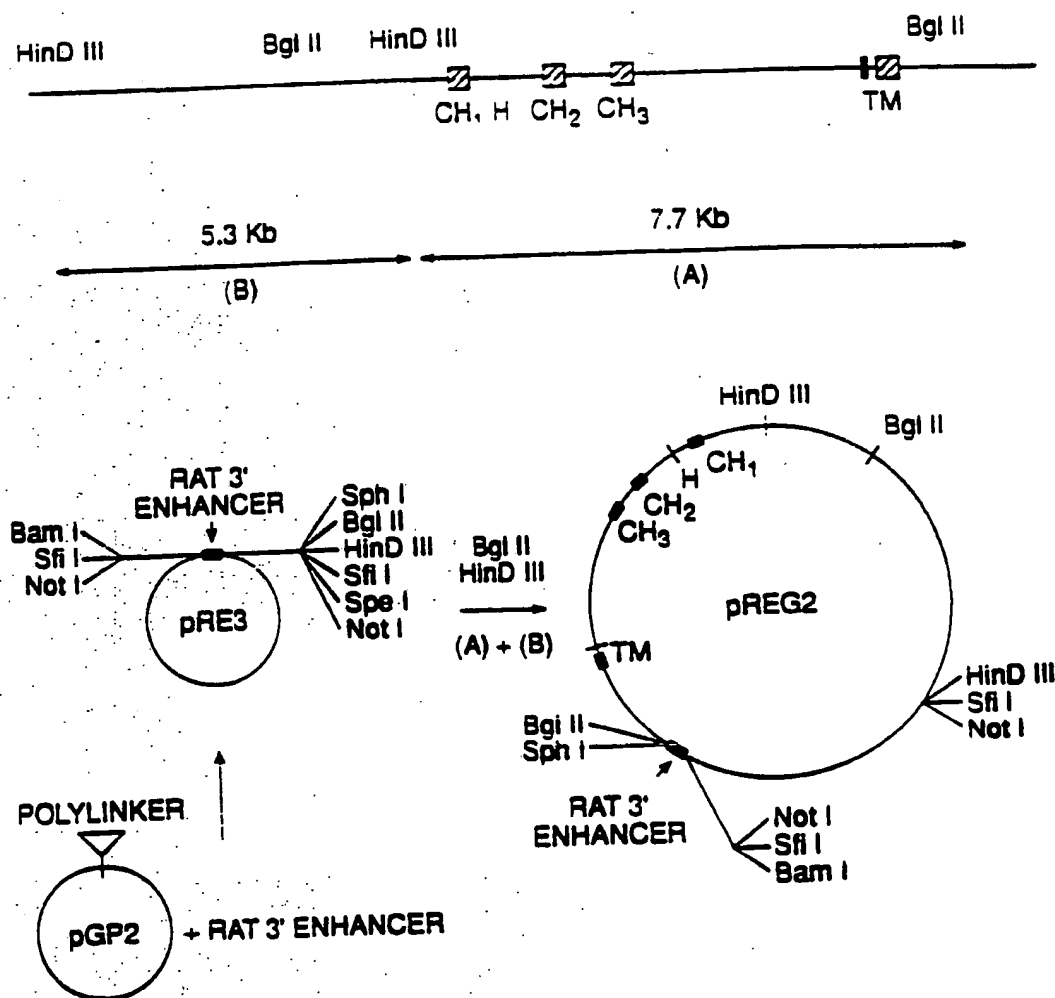
HUMAN C_{γ1} GENE

FIGURE 11

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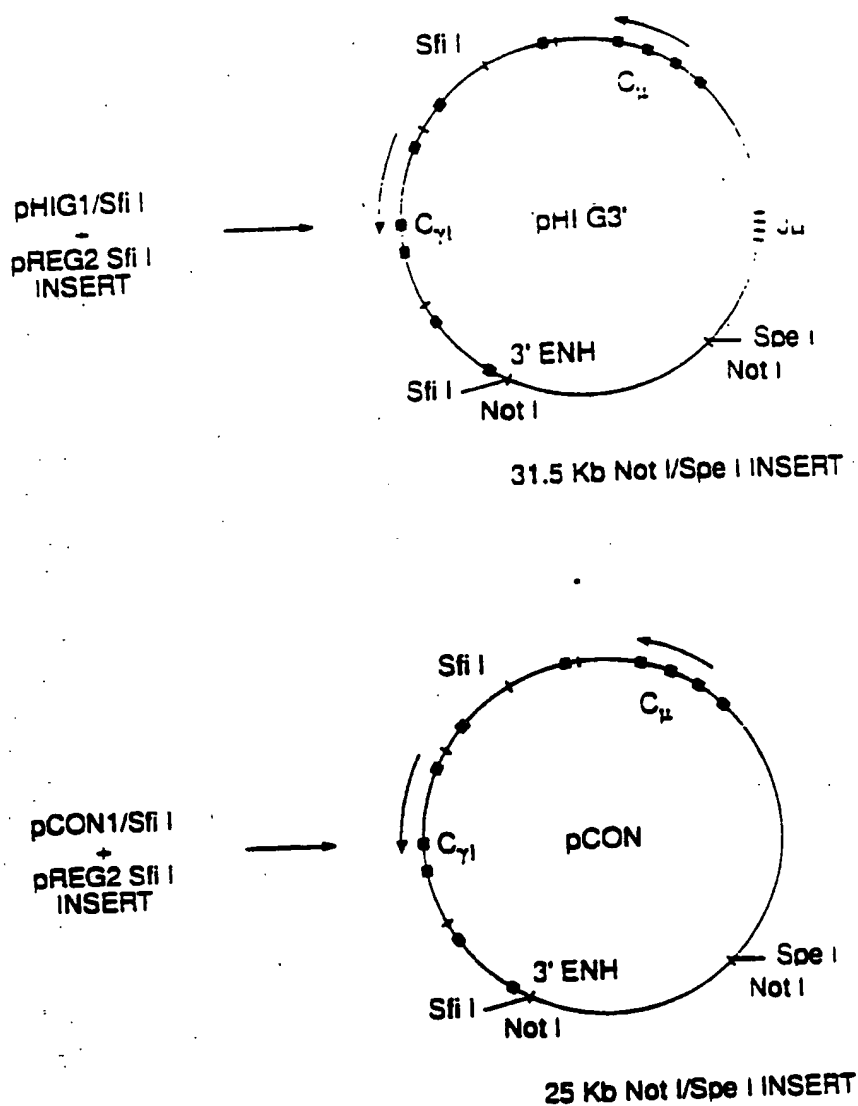


FIGURE 12

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HUMAN D REGION

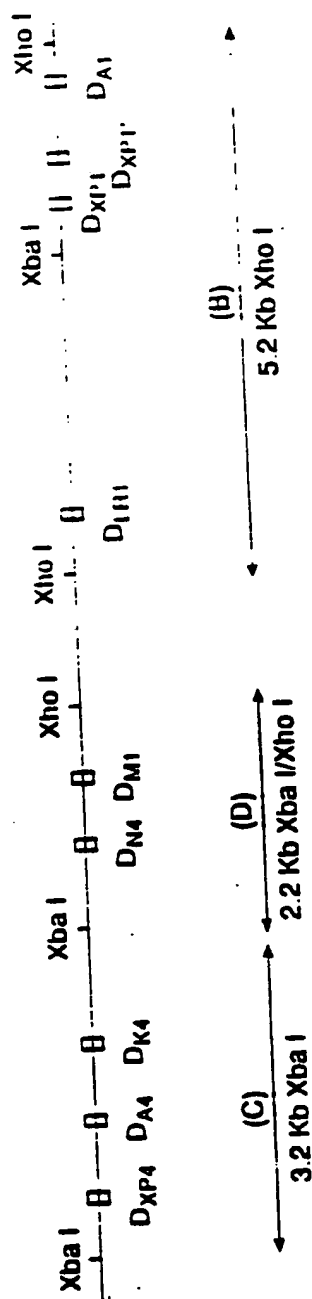


FIGURE 13

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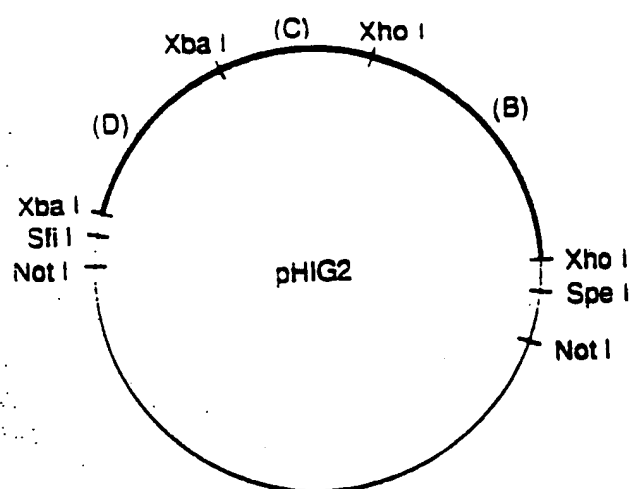


FIGURE 14

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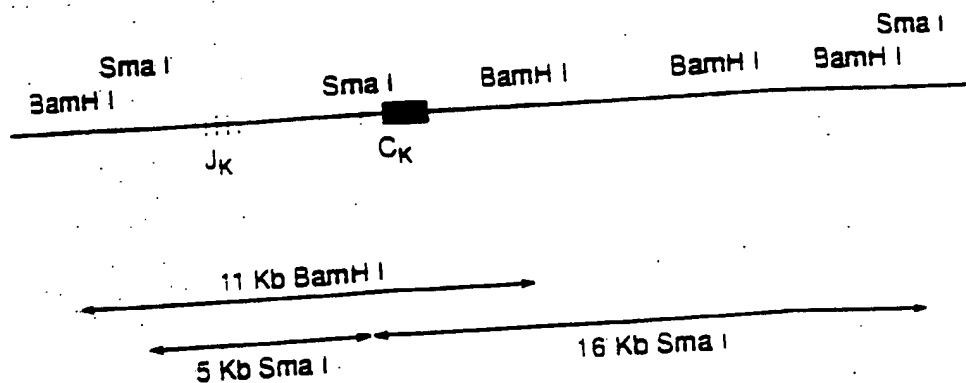


FIGURE 15

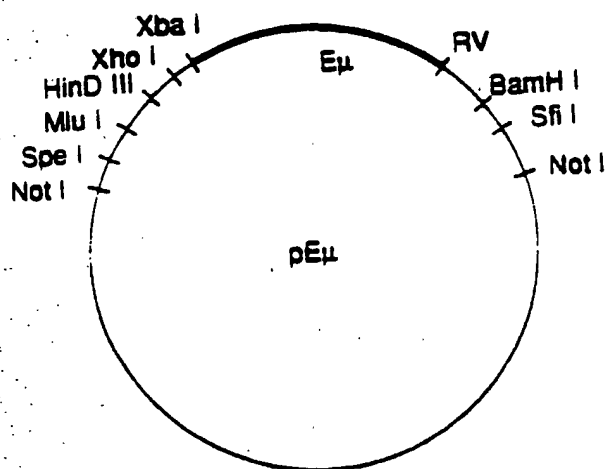


FIGURE 16

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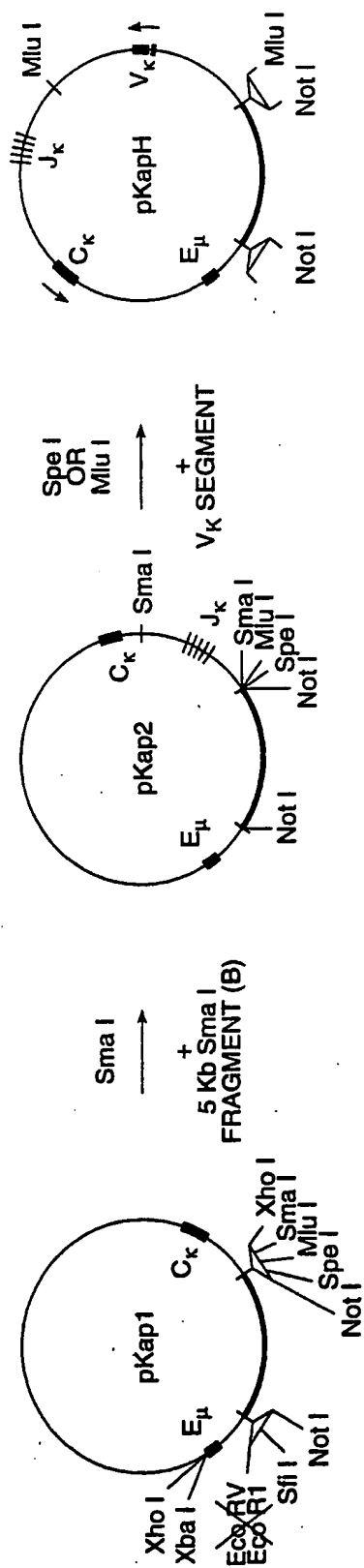


FIG. 17

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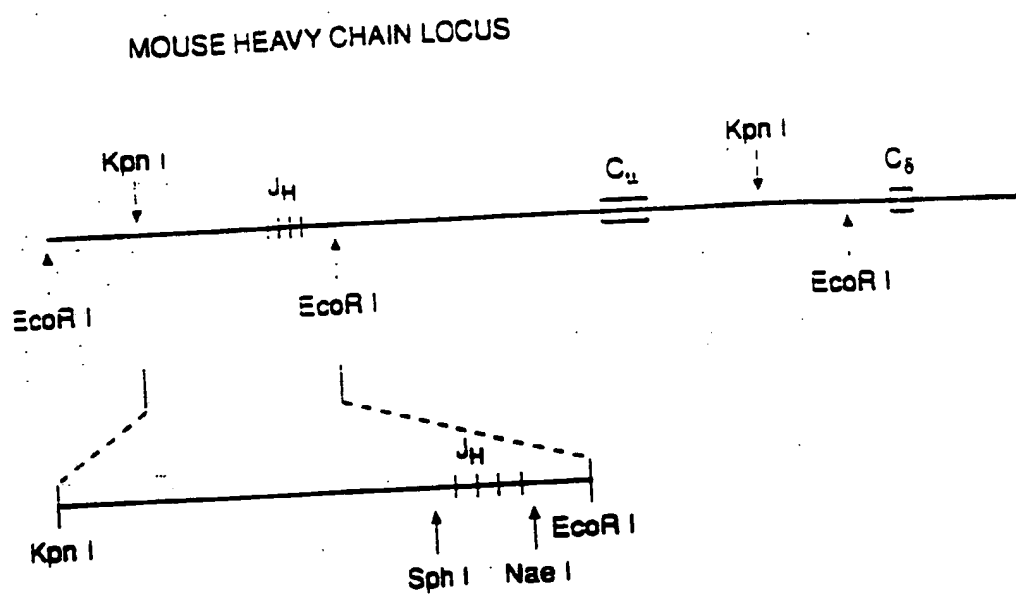


FIGURE 18a

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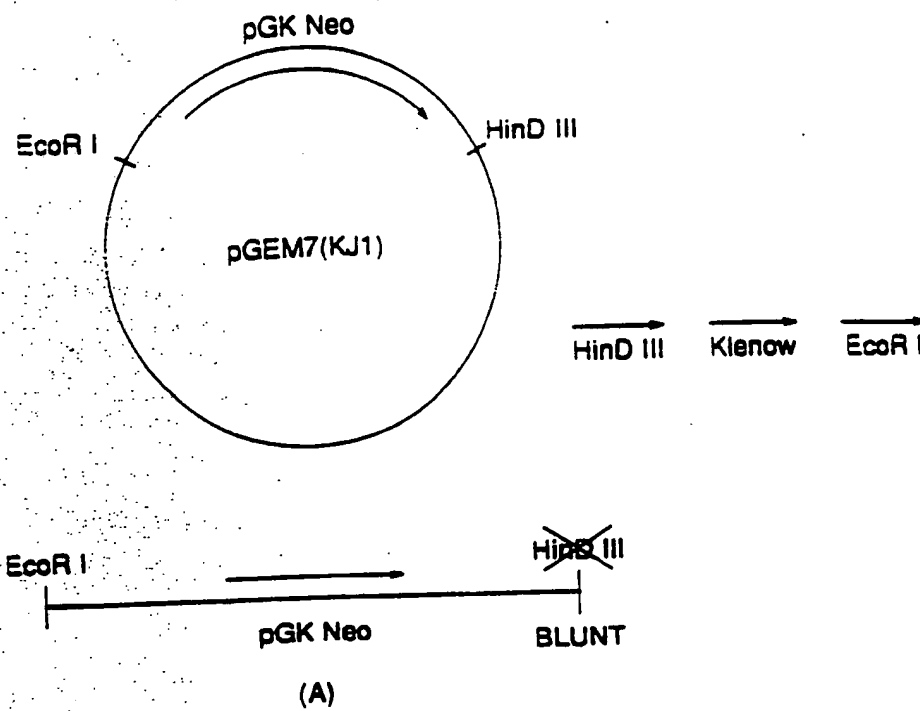


FIGURE 18b

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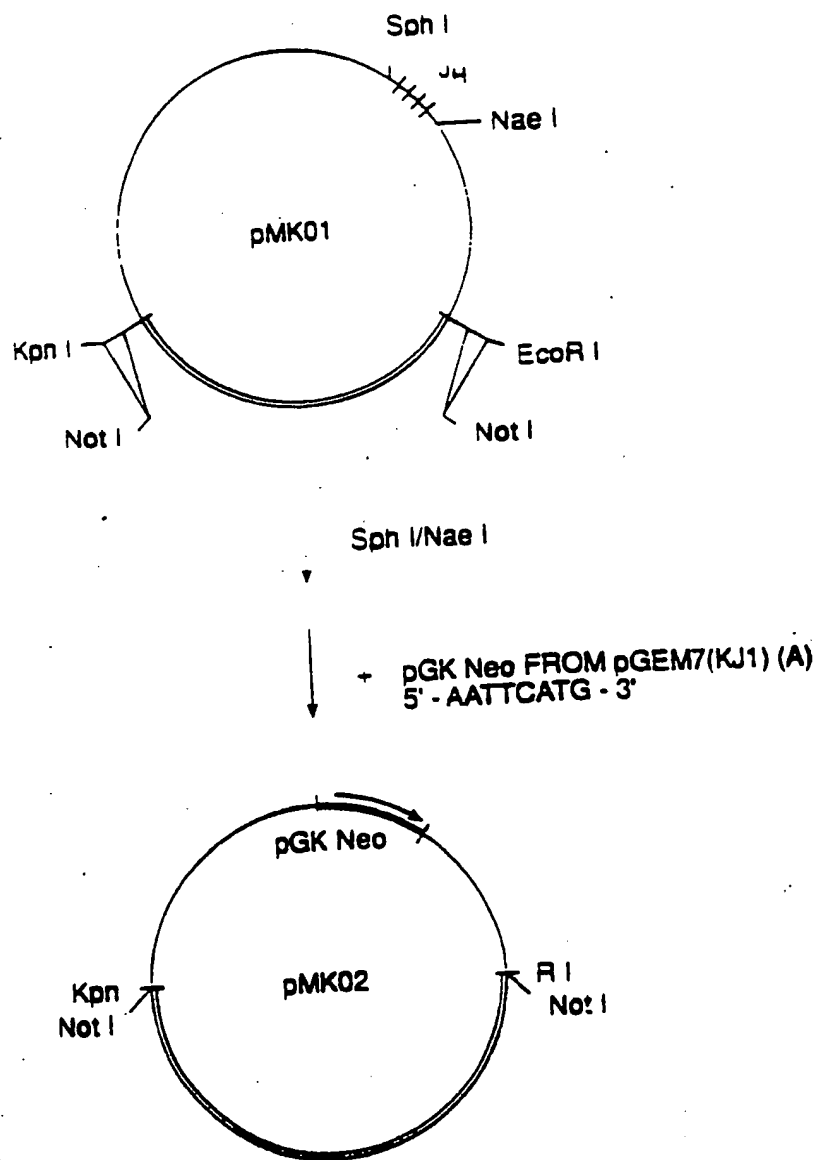


FIGURE 18c

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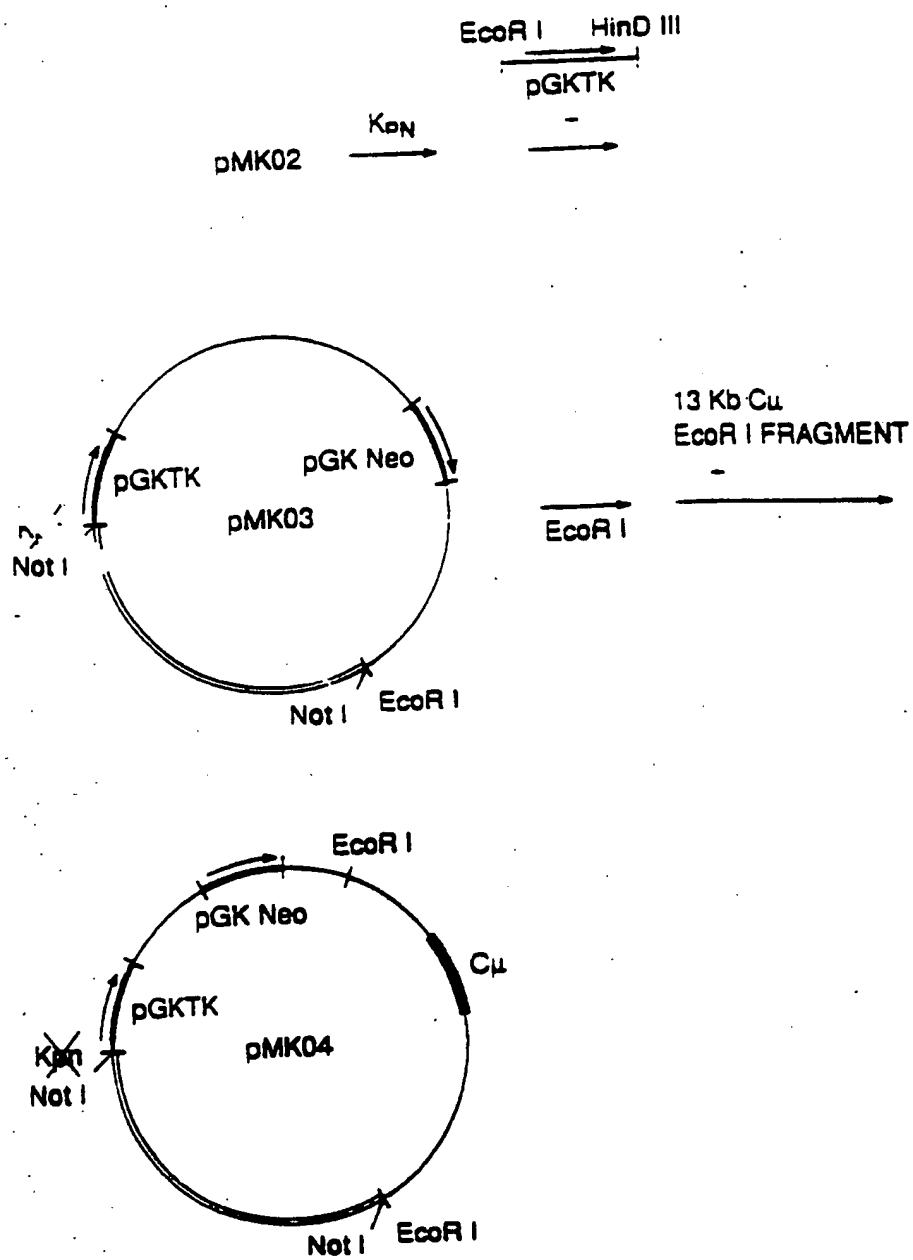


FIGURE 18d

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MOUSE KAPPA GENE

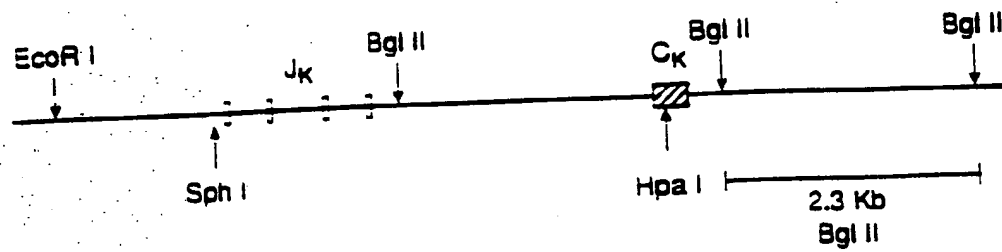


FIGURE 19a

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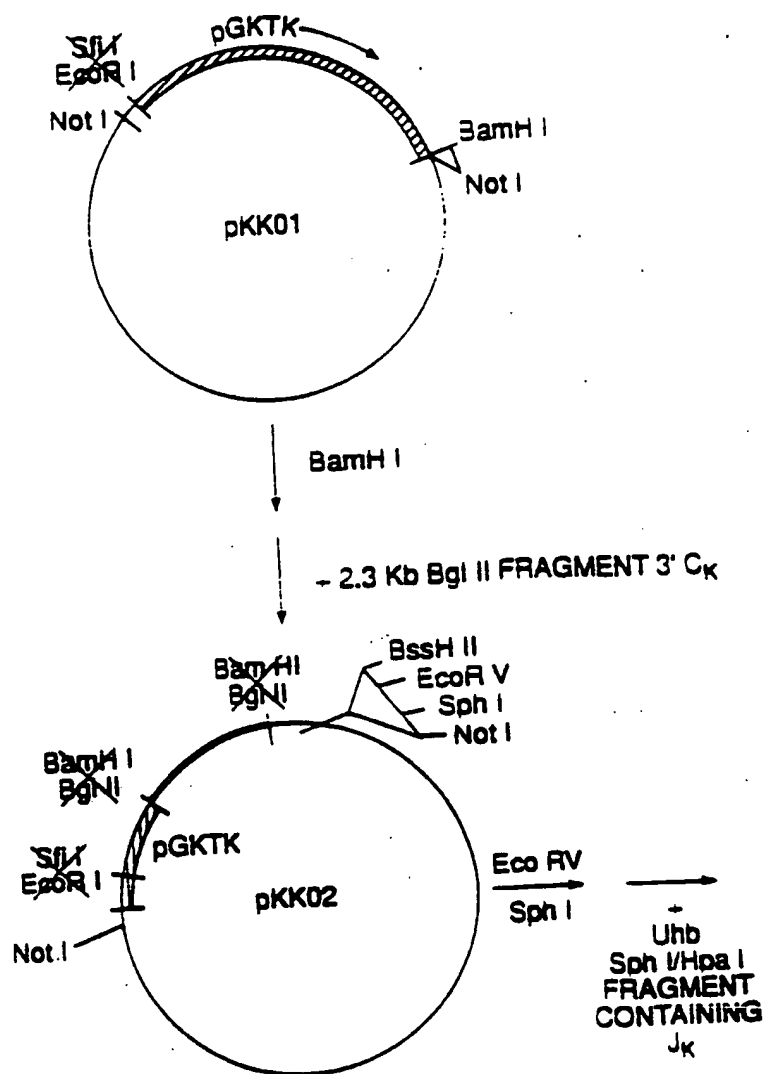


FIGURE 19b

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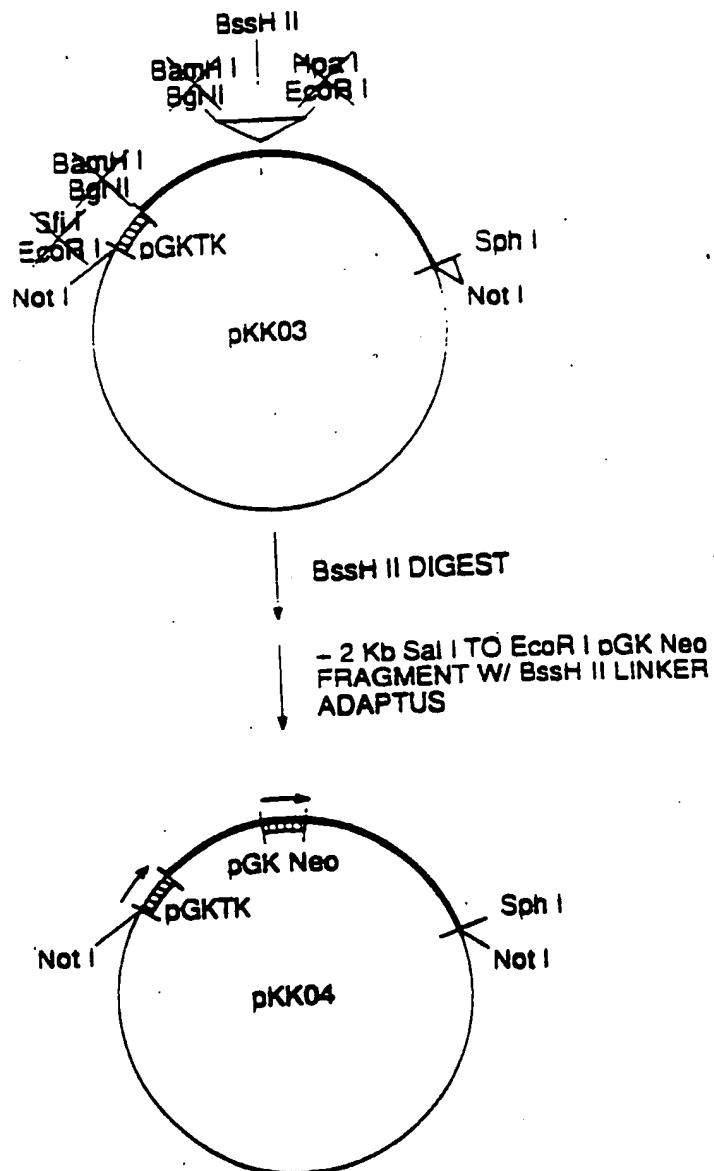
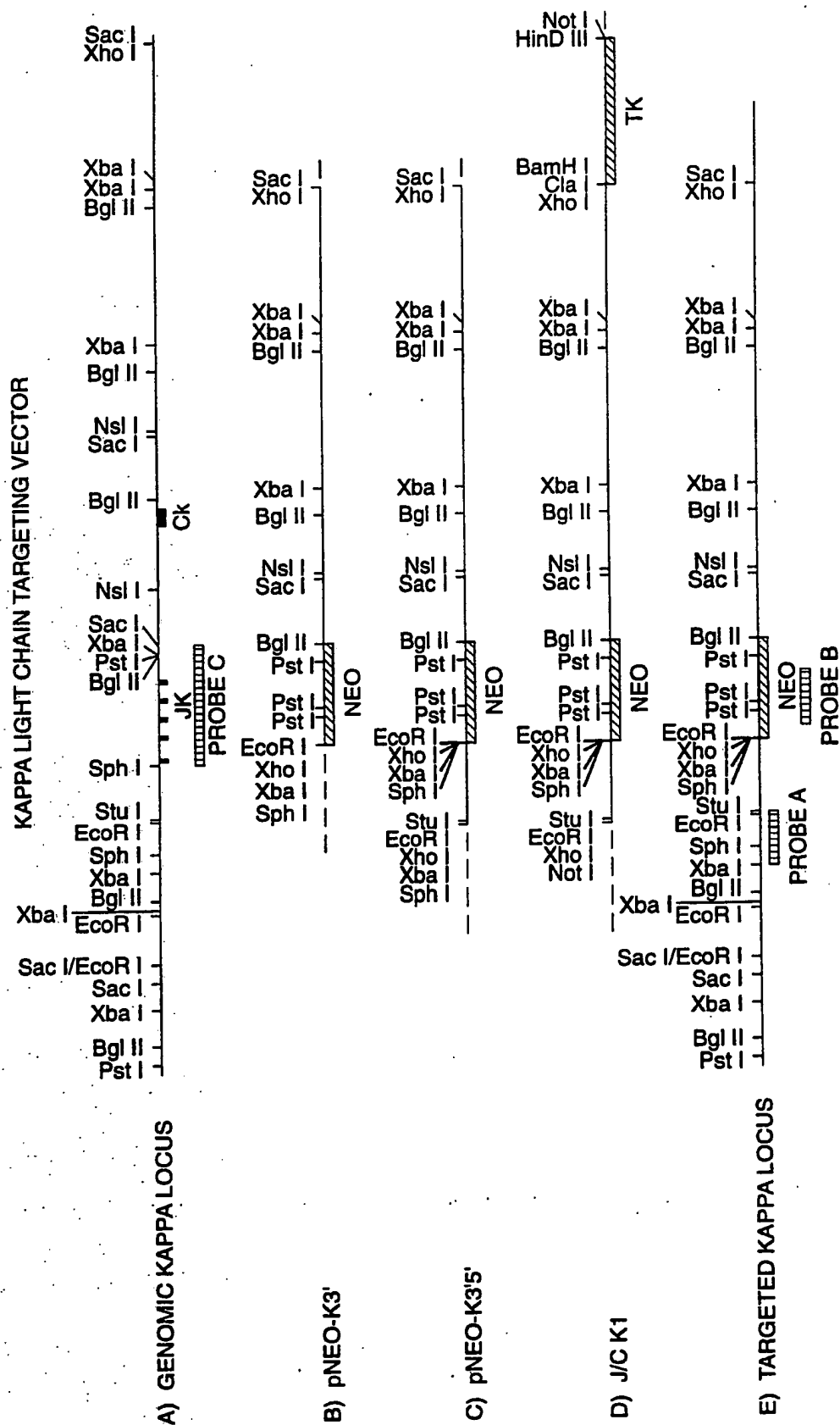
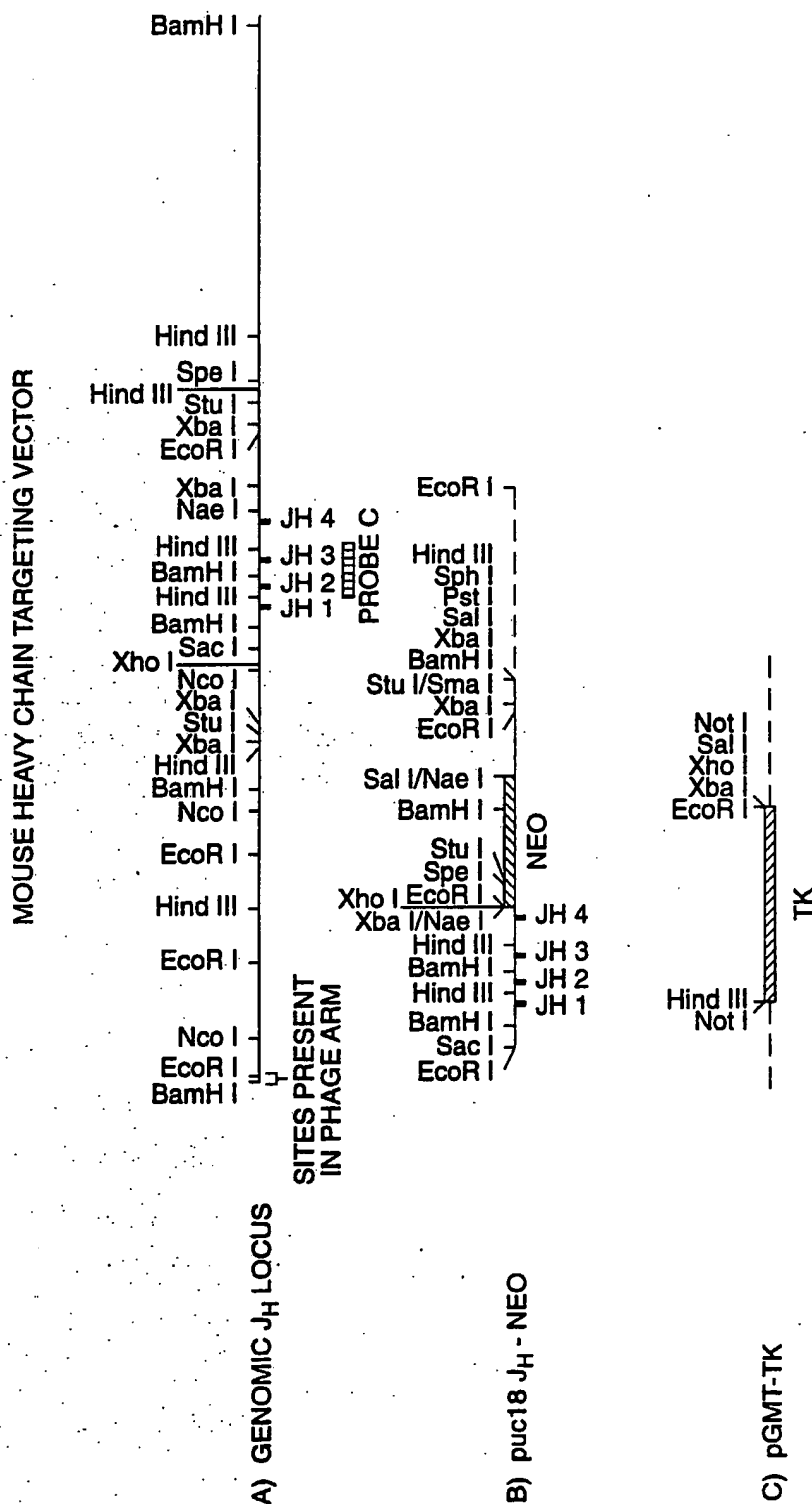


FIGURE 19c



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SUBSTITUTE SHEET

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MOUSE HEAVY CHAIN TARGETING VECTOR

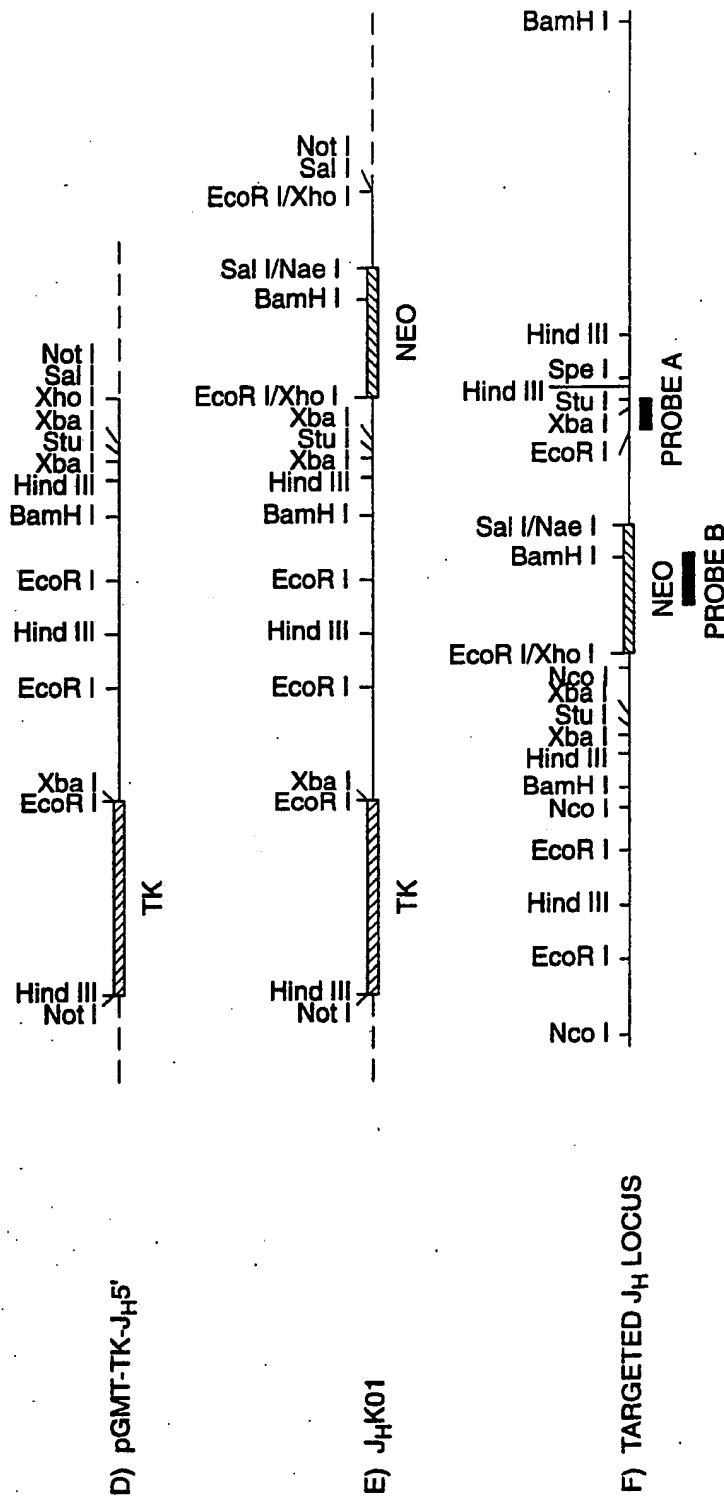


FIG. 21b

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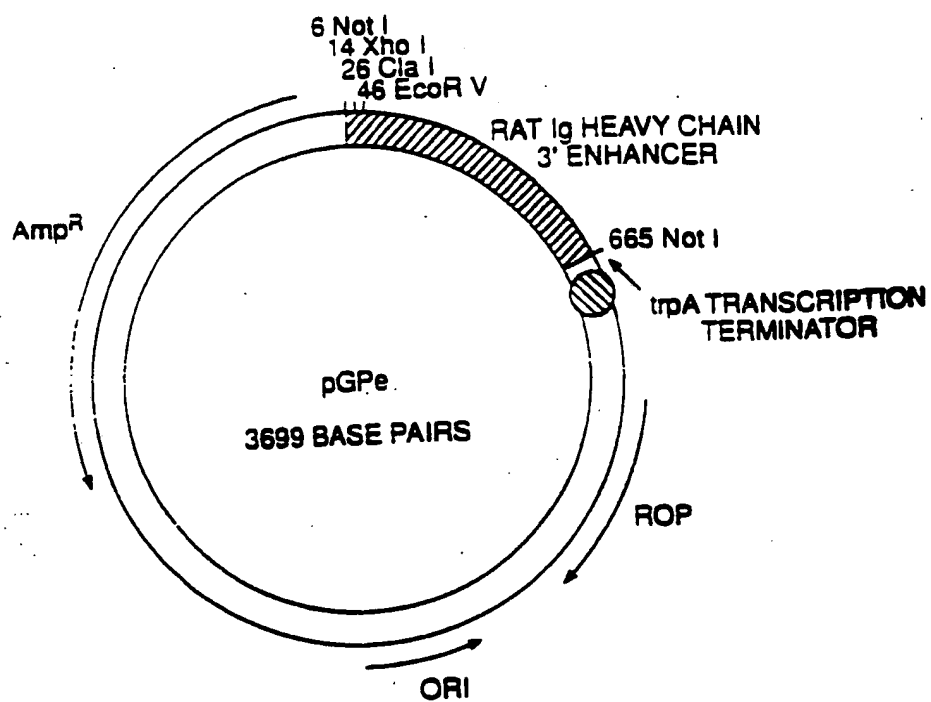


FIGURE 22

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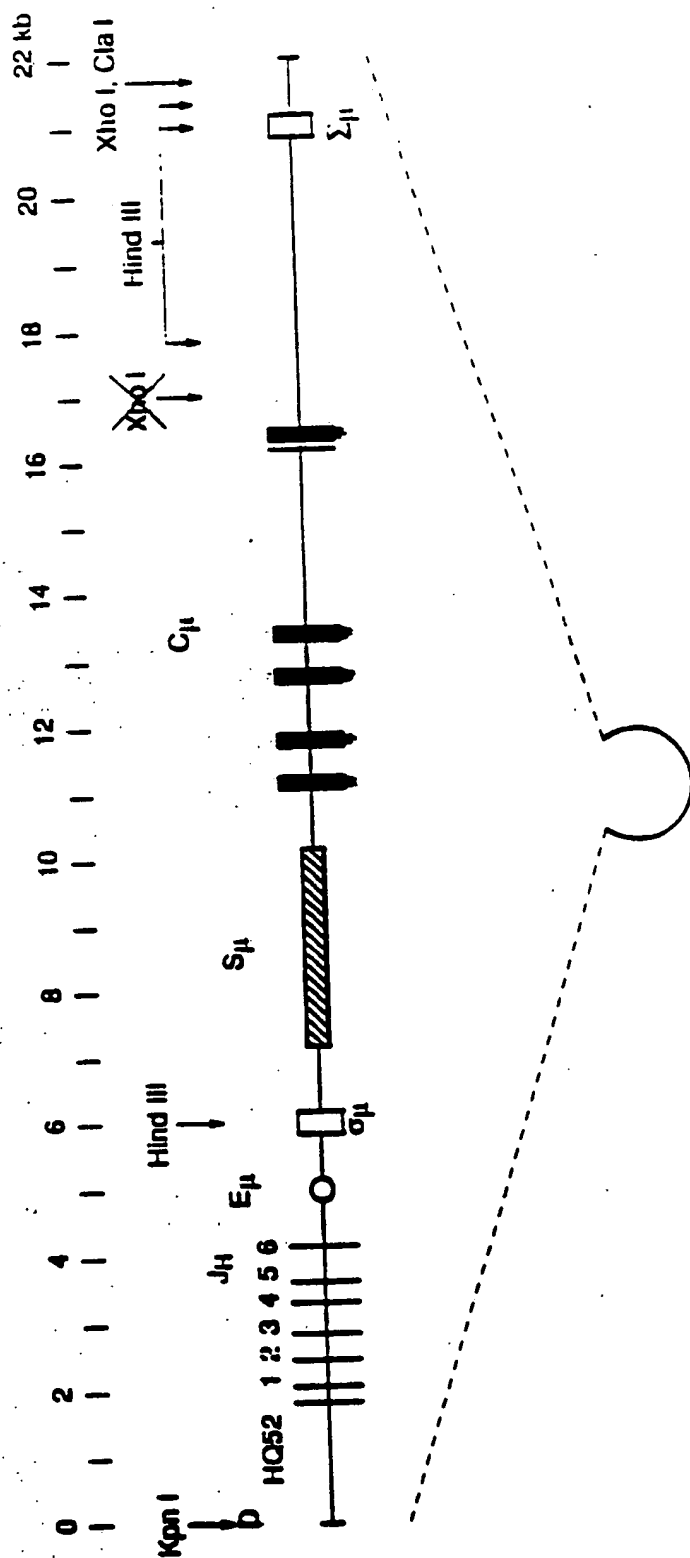


FIGURE 23

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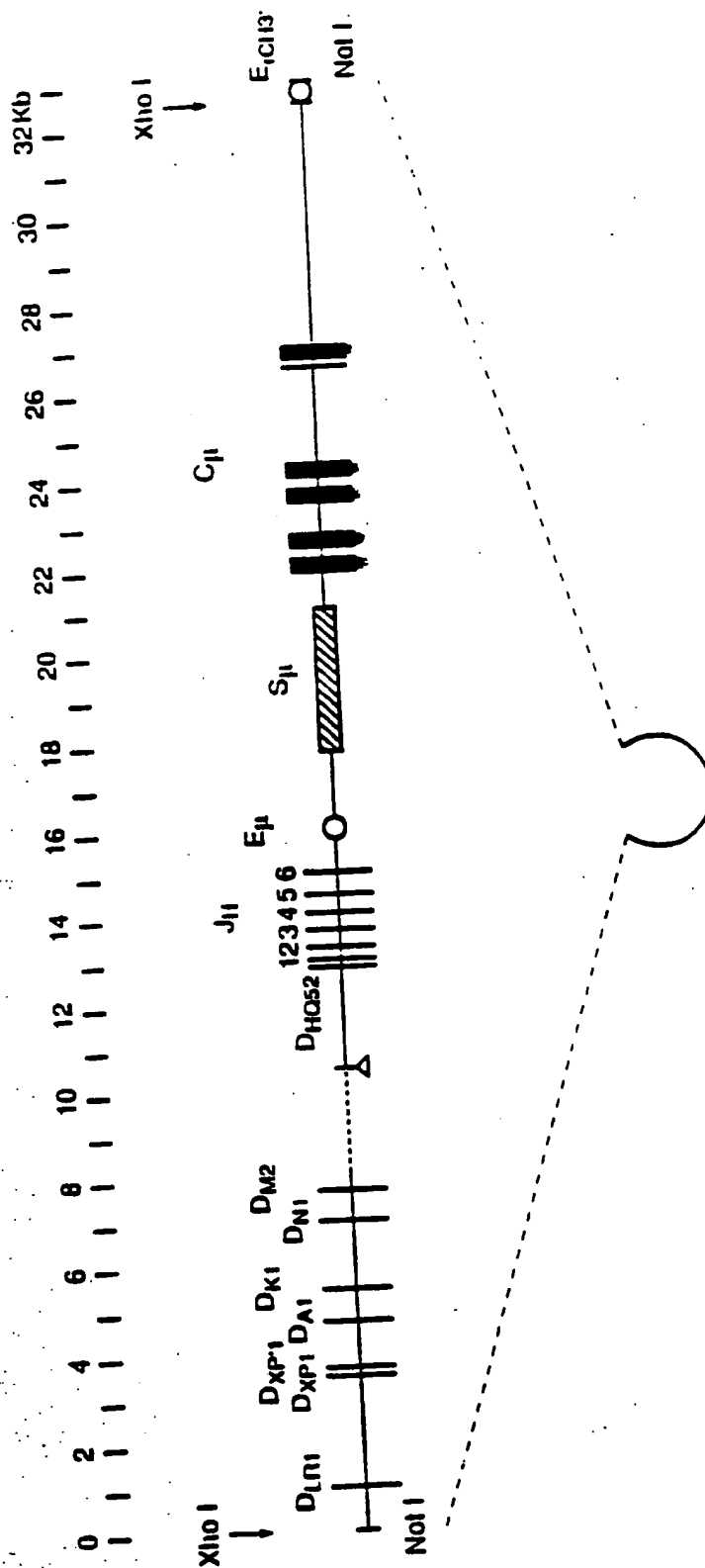


FIGURE 24

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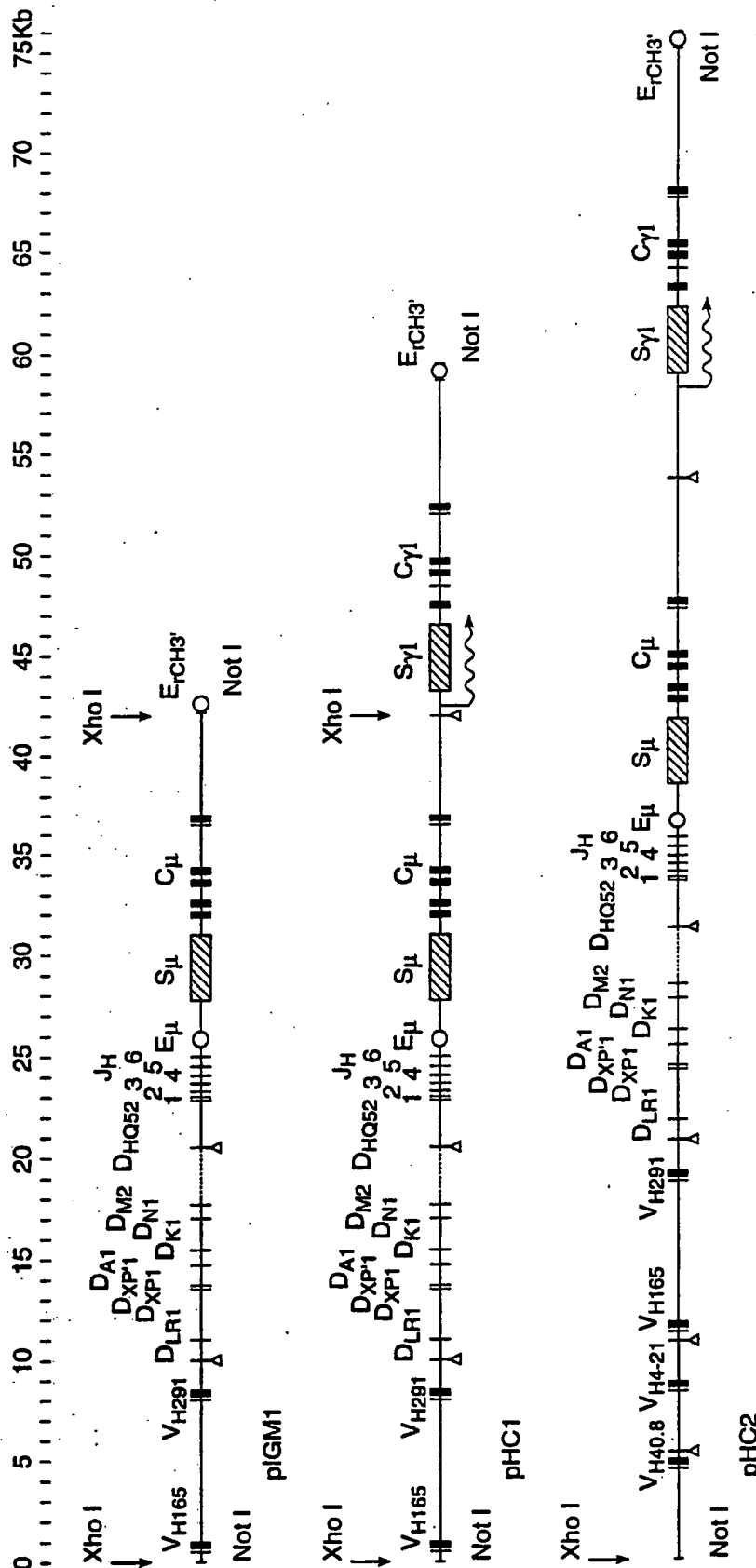


FIG. 25

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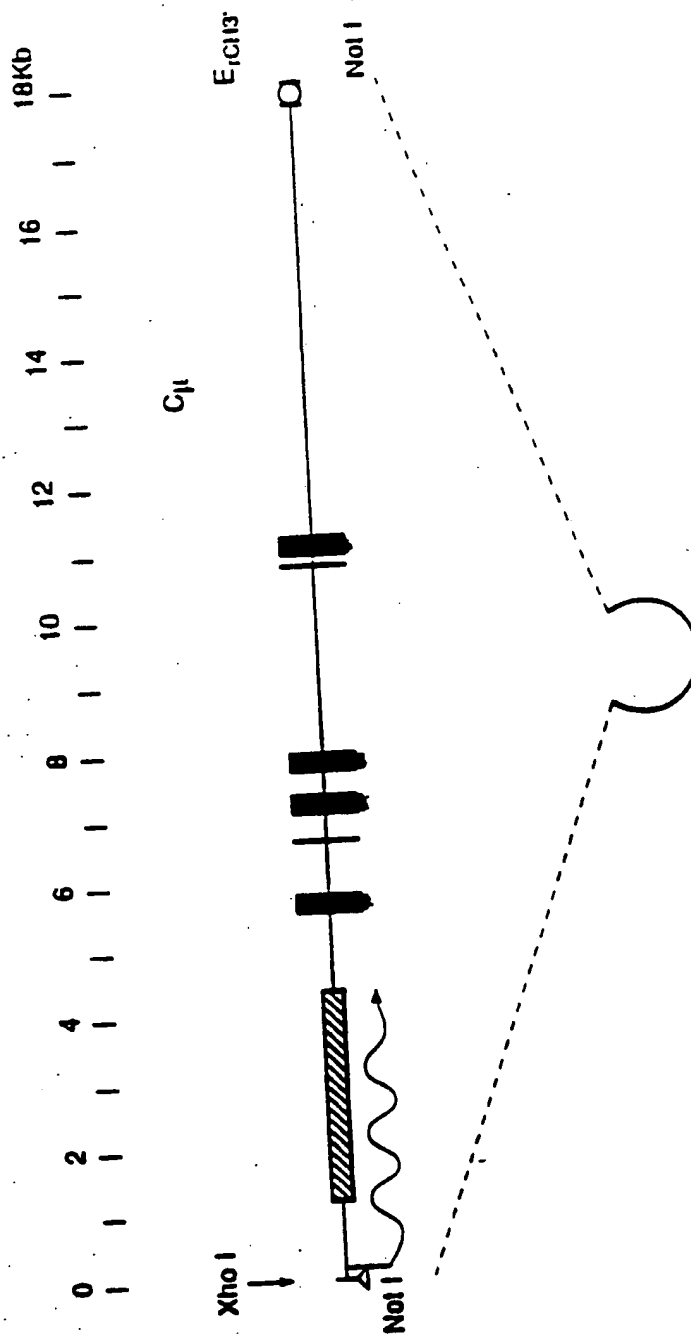


FIGURE 26

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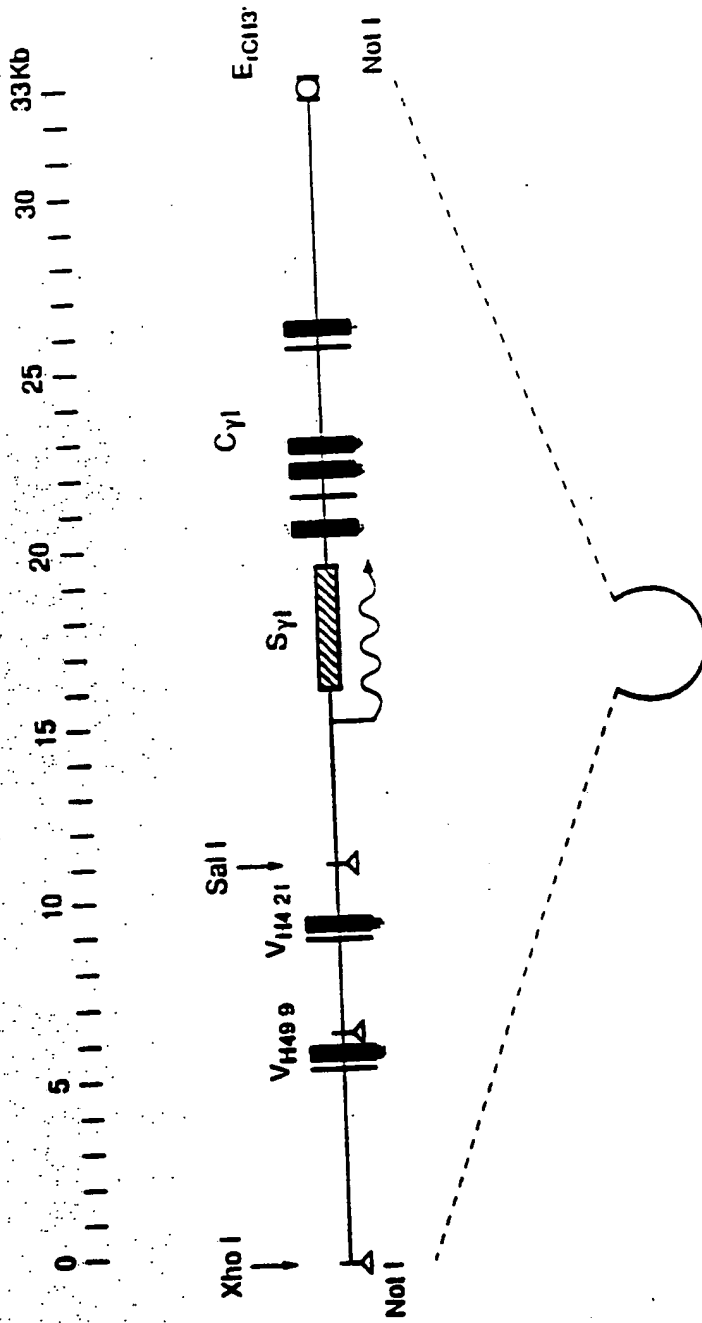
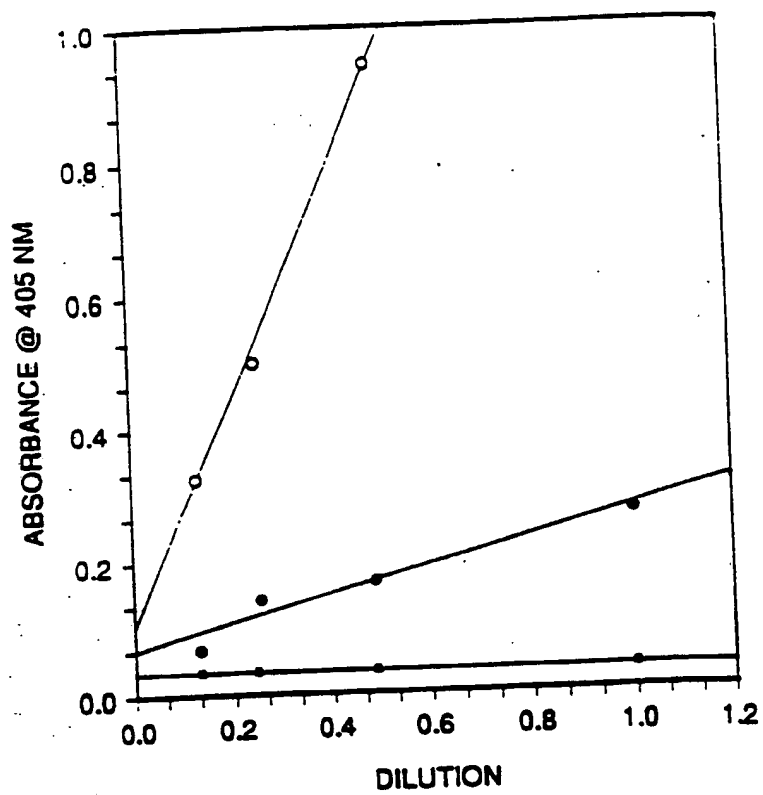


FIGURE 27

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○ IgM } pHC1 TRANSGENIC
● IgG1 }
× IgM } NON-TRANSGENIC CONTROL
+ IgG1 }

FIGURE 28

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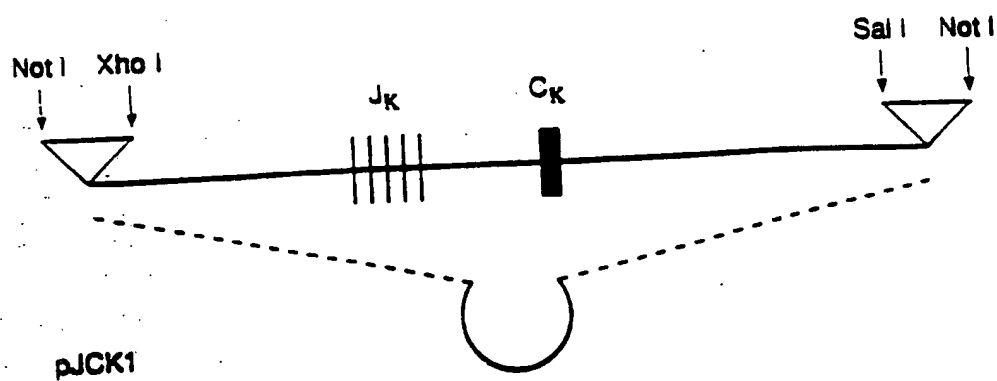


FIGURE 29

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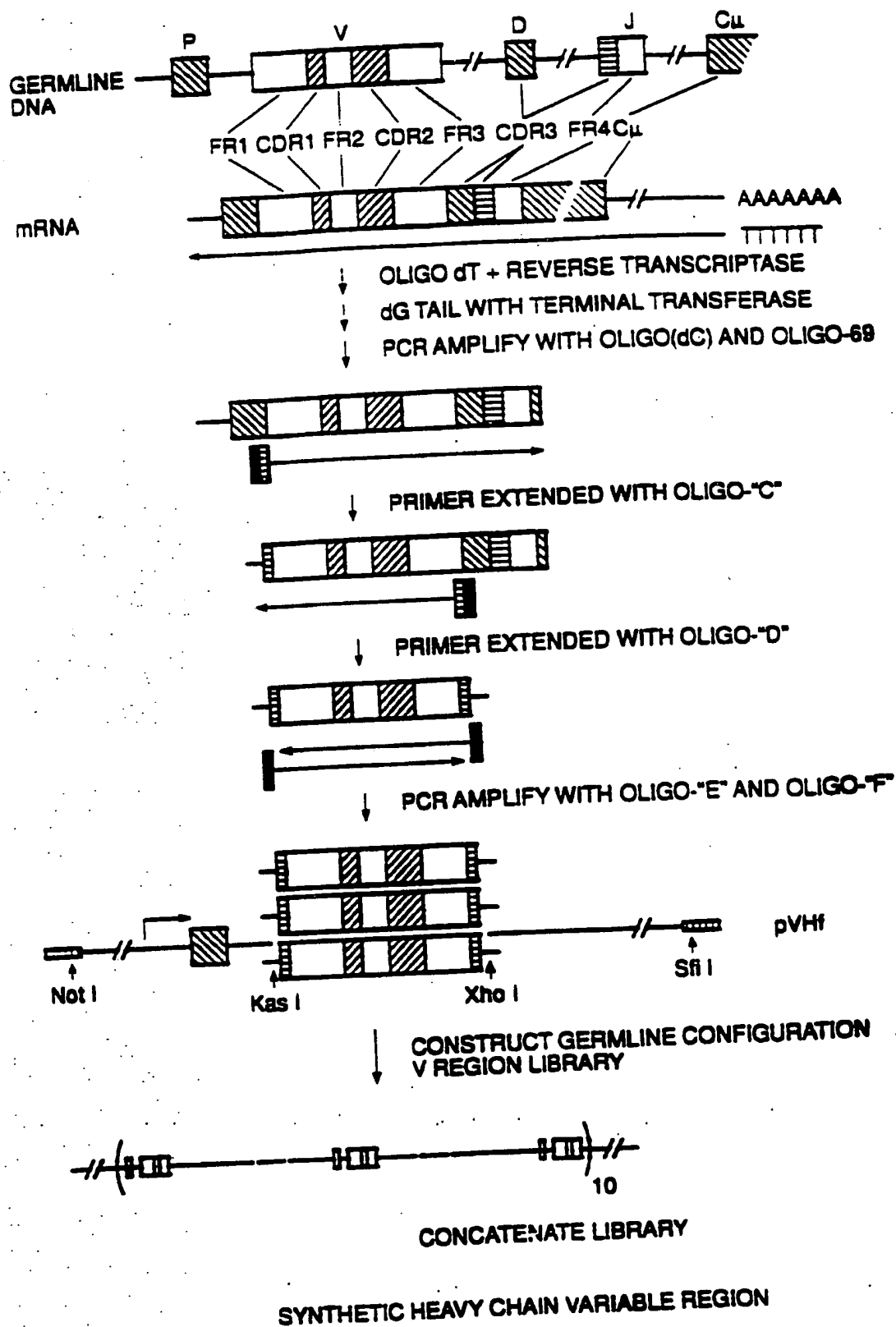


FIGURE 30

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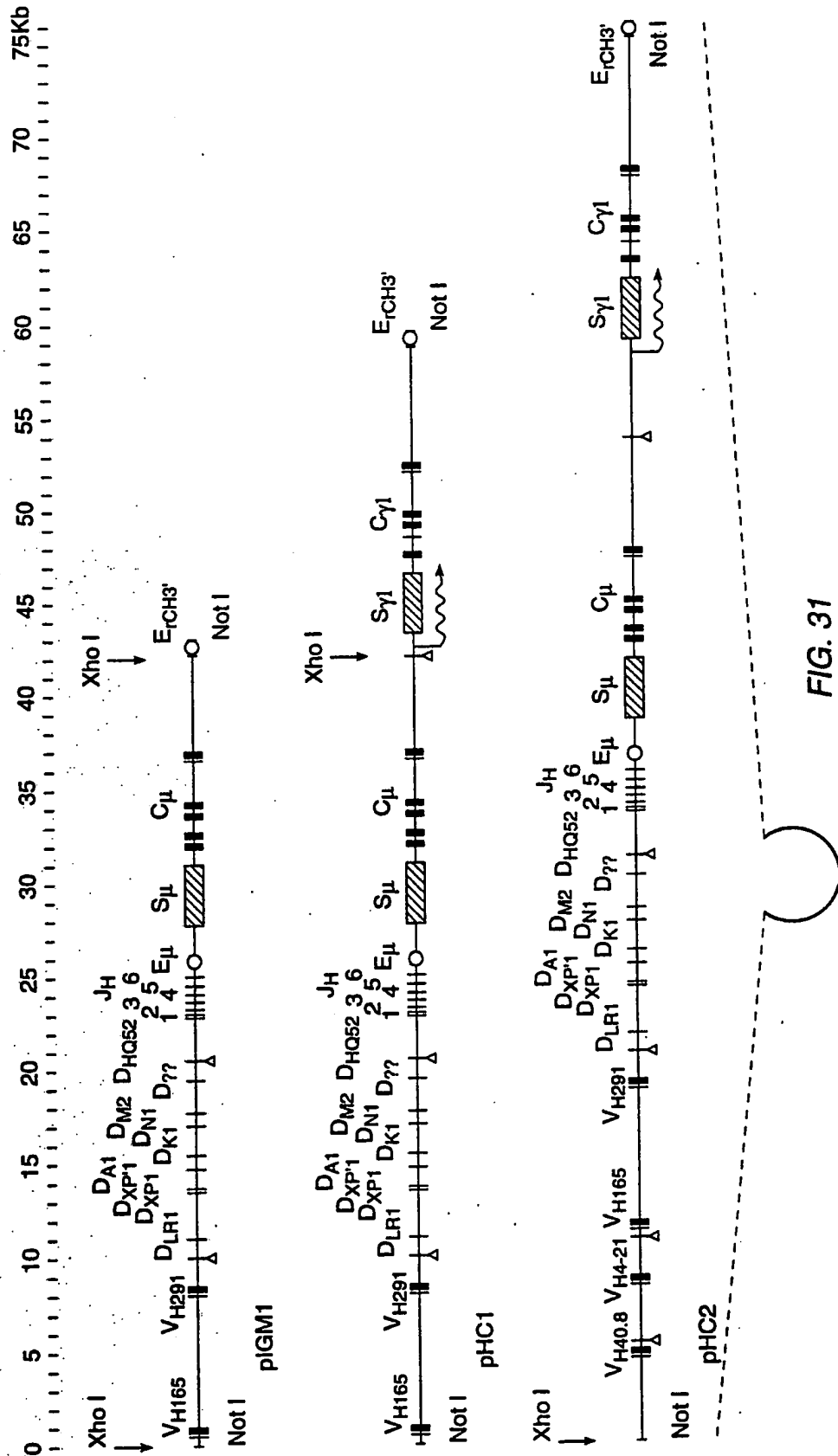
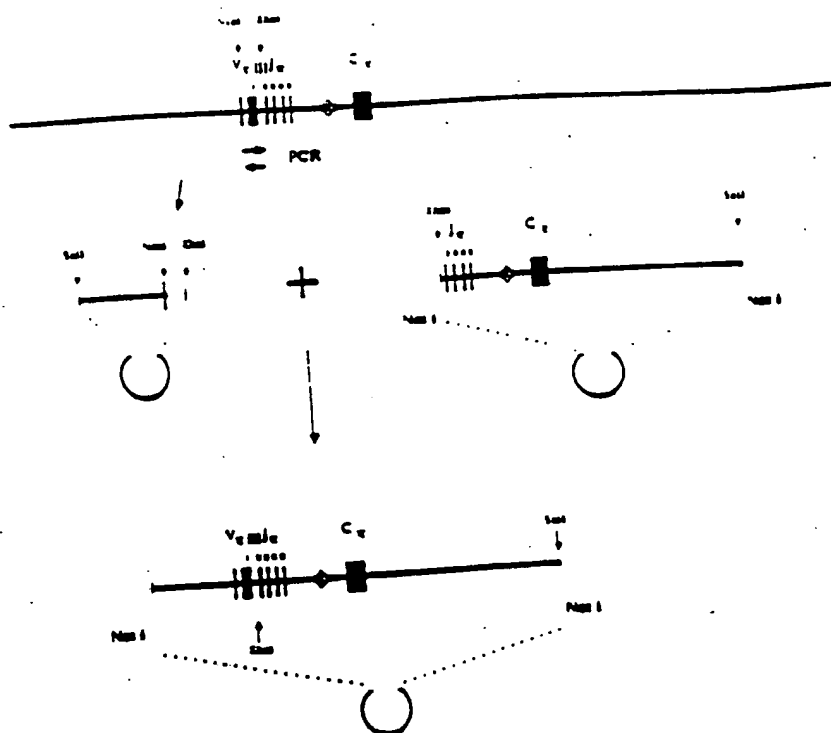


FIG. 31

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Expression cassette for rearranged κ light chain genes.

FIGURE 33

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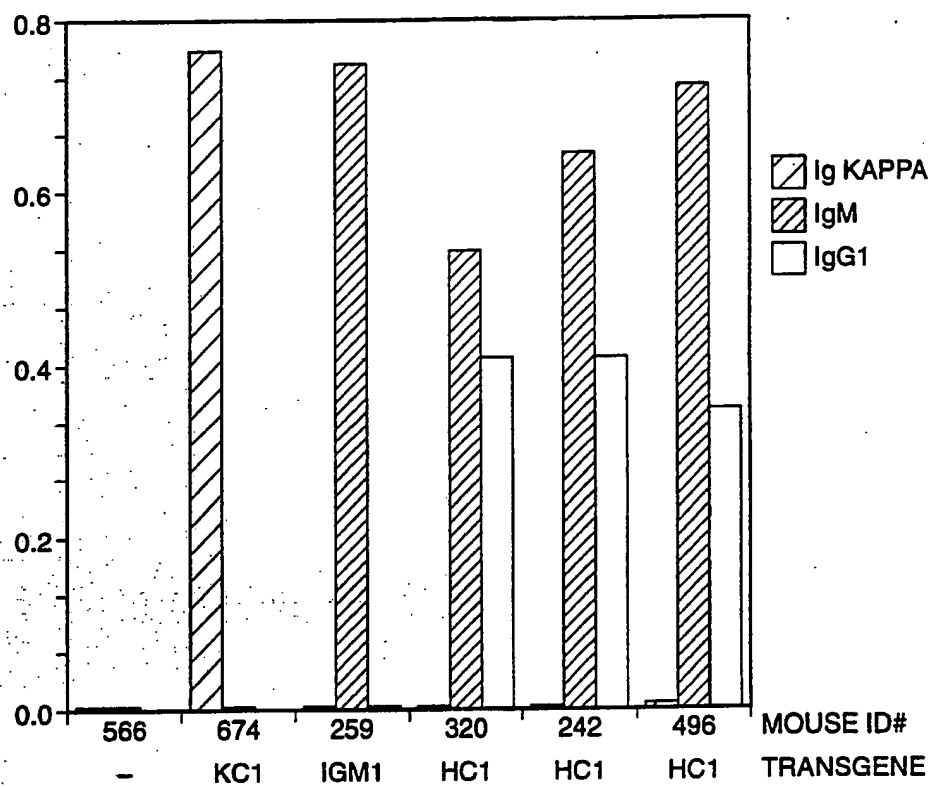


FIG. 34

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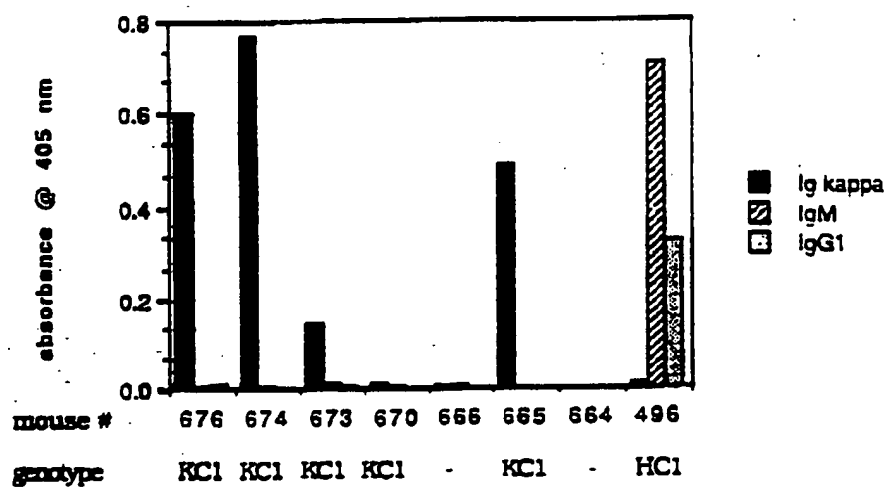


FIGURE 35

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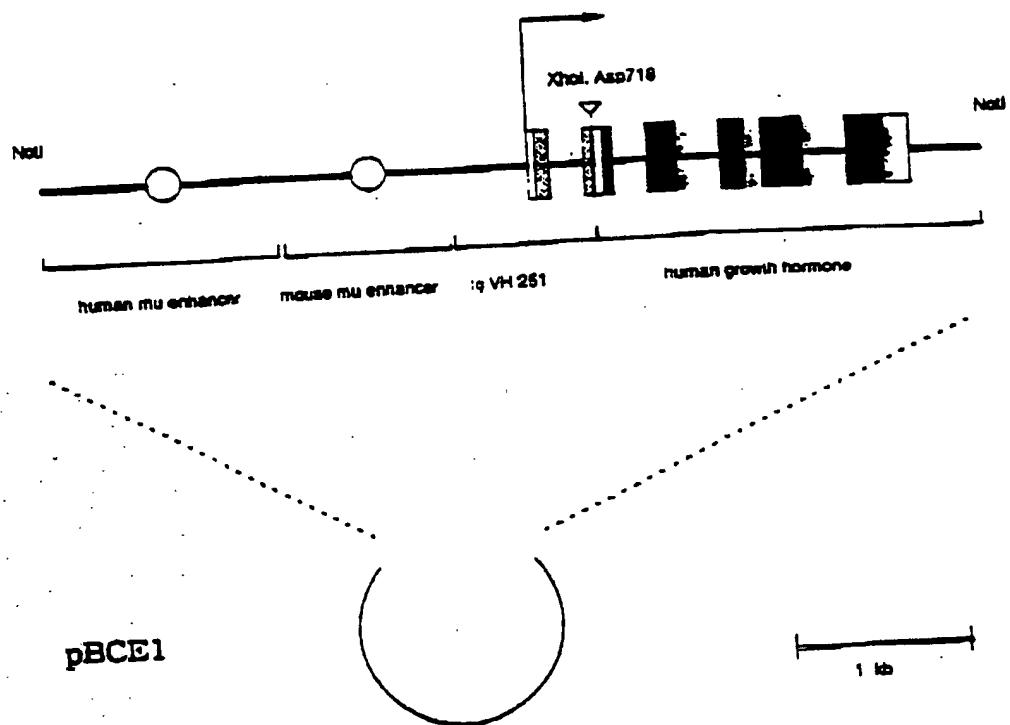


FIGURE 36

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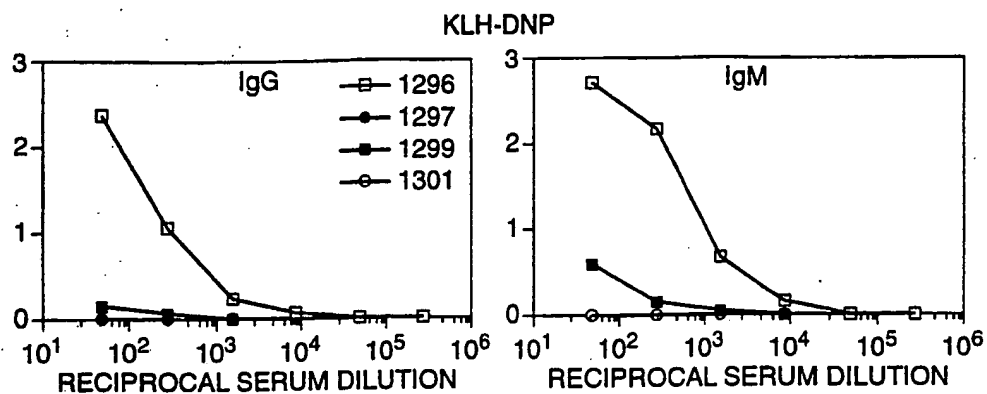


FIG. 37a

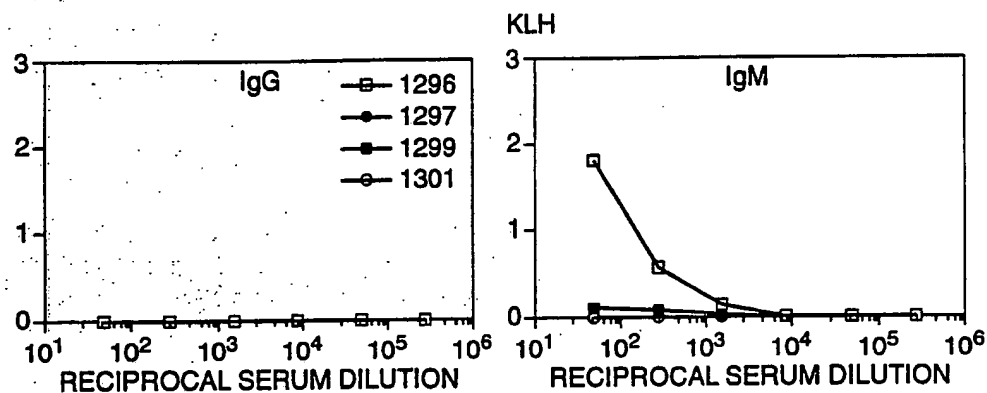


FIG. 37b

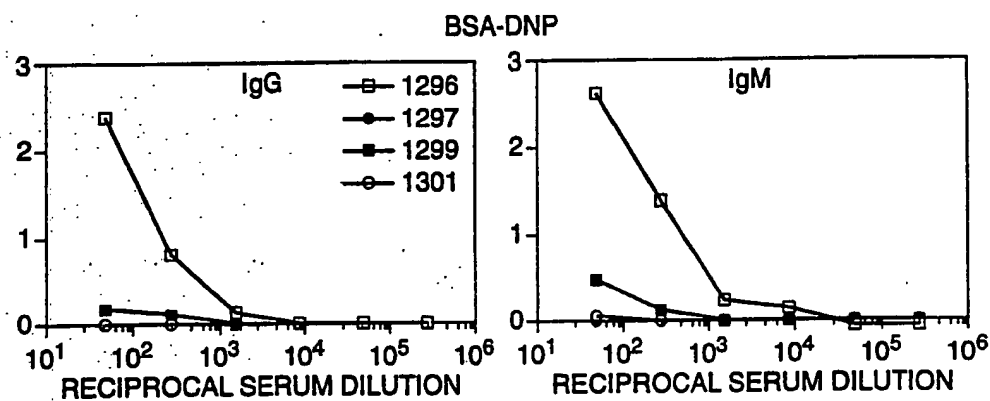


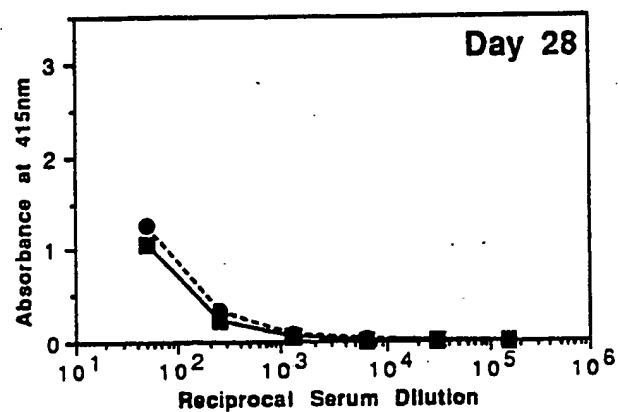
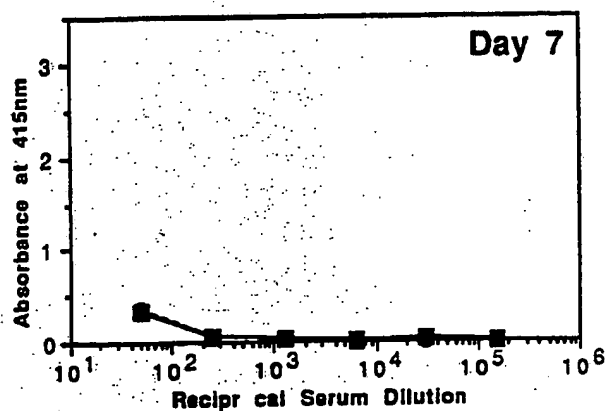
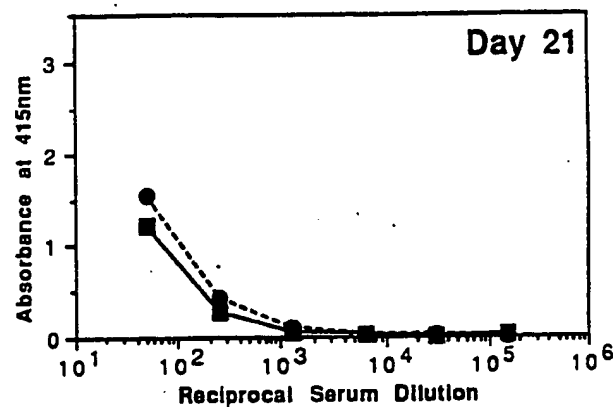
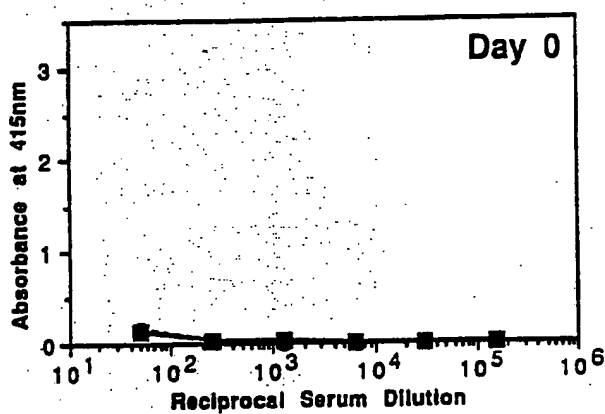
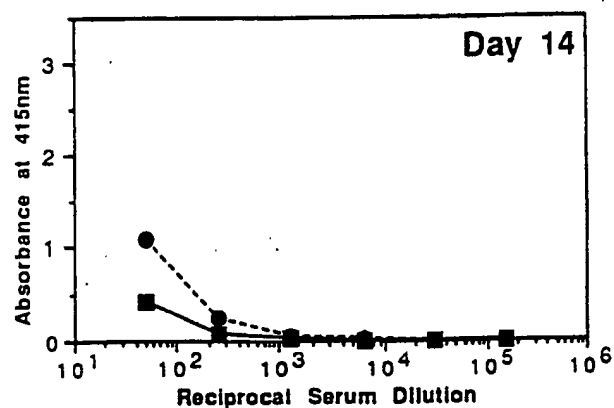
FIG. 37c

SUBSTITUTE SHEET

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FIGURE 38

- HC1-26 human Ig minilocus transgene
(pooled serum from 3 mice)
- -●- - HC1-57 human Ig minilocus transgene
(pooled serum from 3 mice)

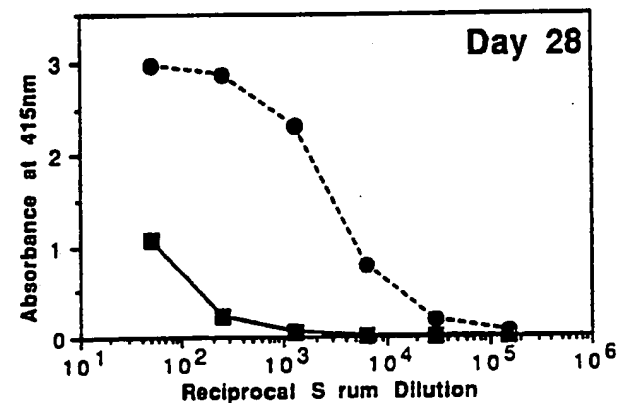
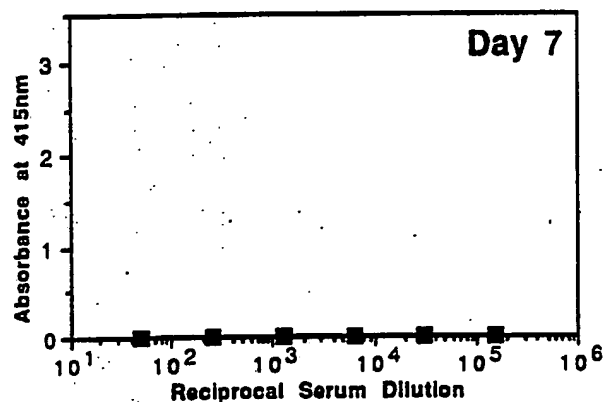
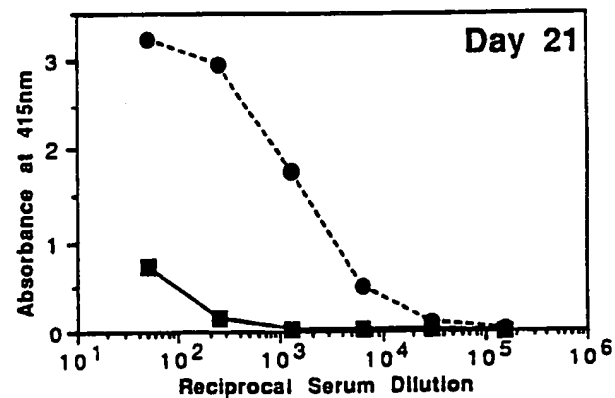
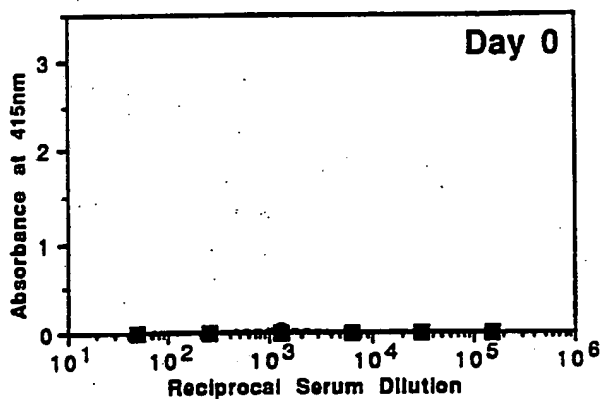
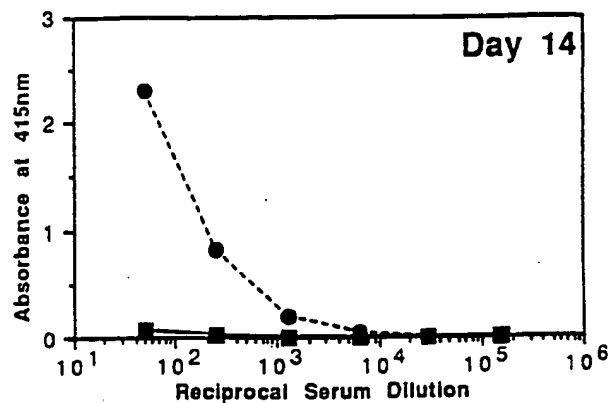


Human Mu heavy chain responses to human CEA determined by ELISA

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FIGURE 39

- HC1-26 human Ig minilocus transgene
(pooled serum from 3 mice)
- HC1-57 human Ig minilocus transgene
(pooled serum from 3 mice)



Human Gamma heavy chain responses to human CEA determined by ELISA

G.L. ²⁰ TCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTT³⁰ ACCAGCTACTGGAT
 S L K X S C K G S G Y S F T S V N X

32.C.....
 5.C.....
 33.
 10.
 24.
 34.
 32.C.P.T.....
 1.T...T...GA.....
 S D
 2.
 3.
 6.T...G.....
 S
 23.
 30.
 4.C.A.....
 Q
 11.A.....
 N
 17.
 27.
 19.G.....T...T.....
 R S
 34.T...A...T.T.....
 V L
 36.
 35.
 25.A.....C.....
 N T
 35.
 36.
 18.A...T...C.....
 N X T
 22.C...T.....
 T
 28.G...A.....
 A N
 33.

CDR I

FIG. 40a

SUBSTITUTE SHEET

CGGCTGGGTGCGCCAGATGCCCGGGAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGT
G W V R Q N P G K G L E W N G X X V P G

**.GG
N**

C
A

X.A

C
A

• T
• F
• T
• F

CDR I

CDR II

FIG. 40b

SUBSTITUTE SHEET

60
GACTCTGATACCAGATACAGCCCGTCCTTCCAAGGCCAGGTC
D S D T R Y S P S F Q G Q V

70
ACCATCTCAGC
T X S A

.....G.....
R

.....

.....

.....C.....G.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....A.....F.....
E

.....

.....

.....

.....

.....

.....

.....

.....

.....C.....
P

.....

CDR II

FIG. 40c

SUBSTITUTE SHEET

CGACAAGTCCATCAGCACCGCCTACCTG⁸⁰CAGTGGAGCAGCCTGAAGGCCTCGGACACCGCC⁹⁰
D K S X S T A Y L Q W S S L K A S D T A

.....CG.....
E

.....G.....GT.T.
R S V

.....A.....T.....A.C.....G.....T.....
N F N T E

.....T.....G.
R

.....T.....

.....G.
V

.....C.
N

.....A.
N

FIG. 40d

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ATGTATTACTGTGCGAGA
N Y Y C A R

.....CAGGGGGGGGATA	TACTGGTAC
Q G G D	Y W Y
	R
	GCT
	A
.....CATTGGctaaAtggggaT	...
N D N D	
.....CGGGattacgatattttgactgggtattatGCG	...
R D A	
	TAC
	Y
.....GtggttcggggaTttattatT	...
V F	
.....GGgtattaTtatgAttcggggaCttattataagtctacCC	.
G D T	
.....ctaactggCCT	.
G L	
.....CATCTT	...
N L	
.....CATCTT	...
N L	
..T.....CATCTT	...
X N L	
.....CG.....CATCTT	...
R N L	
.....CAAGG	
Q G	
.....CAAAC	
Q T	
.....CATggatatagcagcagctggtacGTGGTCCGACCCCA	
N W F R P L	
.....GCCgtataCcagcagctggtT	
A T F	
.....CAGGGC	...
Q G	
.....CAAAGGGG	...
Q R G	
.....GGGATCGTGG	...
G S W	
	AACTGG
	N W
.....CTCCCAATGACAGT	...
L P N D S	
.....CGGGGGtactatggttcggggagttattat
R G	
	TACTACTACTACTACGGT
	Y Y Y Y Y G
.....CATGagcagctggtacAGGGT
N E Q G	
.....GATATGGGGGGGGCCTCT.....T.....
D N G G A SF.....F.....
	C..C..G.....
	H H D
.....CG
R	

FIG. 40e

CDR III

SUBSTITUTE SHEET

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TTCGATCTCTGGGGCCGTGGCACCTGGTCACTGTCTCCTCAG
P D L W G R G T L V T V S S

TTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG
F D X W G Q G T N V T V S S

TTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG
F D Y N G Q G T L V T V S S

.....T.....

....A.
N

.....A
Q
A
Q
A
Q
A
Q

.....G P G N P G H R L L S
.....T
P

TTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG
F D P W G Q G T L V T V S S

ATGGACGTCTGGGGGCAAGGGACCACGGTCACCGTCTCCTCAG
N D V W G Q G T T V T V S S

CDR III

FIG. 40f

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TTTTCTGGCC TGACAACCAG GGTGGCGCAG GATGCTCAGT GCAGAGAGGA 50
 AGAAGCAGGT GGTCTCTGCA GCTGGAAGCT CAGCTOCCAC CCAGCTGCTT 100
 TGCATGTGCC TOCCAGCTGC CCTACCTTCC AGAGOOOATA TCAATGGCTG 150
 TGTCAGAGCC CTGGGGAGGA ACTGCTCAGT TAGGAOCCAG AGGGAAOCAT 200
 GGAAGOOCCA GCTCAGCTTC TCCTCTCTCT GCTACTCTGG CTOCCAGgtg Me 250
 tGluAlaPro AlaGlnLeuL euPheLeuLe uLeuLeuTrp LeuPro
 agggggaacc atgaggtggt tttgcacatt agtgaaaact cttgccacct 300
 ctgctcagca agaaatataa ttaaaattca aagtatatca acaatttttg 350
 ctctactcaa agacagttgg tttgatcttg attacatgag tgcattttctg 400
 ttttatttcc aatttcagAT AOCACCGGAG AAATIGTGT GACACAGTCT 450
 Asp ThrThrGlyG luIleValLe uThrGlnSer
 CCAGCCAOCC TGCTTTTGTC TOCAGGGGAA AGAGOOCAOCC TCTCCTGCAG 500
 ProAlaThrL euSerLeuSe rProGlyGlu ArgAlaThrL euSerCysAr
 GGOCAGTCAG AGTGITAGCA GCTACTTAGC CTGGTACCAA CAGAAOCTG 550
 gAlaSerGln SerValSerS erTyrLeuAl aTrpTyrGln GlnLysProG
 GOCAGGCTCC CAGGCTOCTC ATCTATGATG CATOCCACAG GGOCACITGC 600
 lyGlnAlaPr oArgLeuLeu IleTyrAspA laSerAsnAr gAlaThrGly
 ATCCAGCCA GGITCAGTGG CAGTGGGTCT GGGACAGACT TCACTCTCAC 650
 IleProAlaA rgPheSerGl ySerGlySer GlyThrAspP heThrLeuTh
 CATCAGCAGC CTAGAGCCTG AAGATTTTGC AGTTTATTAC TGTCAGCAGC 700
 rIleSerSer LeuGluProG luAspPheAl aValTyrTyr CysGlnGlnA
 GTAGCAACTG GOCTOCCACA GTGATTCCAC ATGAAACAAA AACOCCAACA 750
 rgSerAsnTr pPro
 AGAOCATCAG TGTTTACTAG ATTATTATAC CAGCTGCTTC CTTTACAGAC 800
 AGCTAGTGGG GT 812

FIGURE 41

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AGGGGGGGGC AGATGCTCAG TGCAGAGAGA AGAAACAGGT GGTCTCTGCA 50
 GCTGGAAGCT CAGCTCCAC CCGAGCTGCT TTGCATGTCC CTOCCAGCTG 100
 CCCTAOCCTTC CAGAGCCCAT ATCAATGCGT GGGTCAGAGC TCTGGGGAGG 150
 AACTGCTCAG TTAGGACCCA GACGGAACCA TGAAGCCCC AGCGCAGCTT 200
 CTCCTCCTCC TGCTACTCTG GCTCAGAGgt gaggggaata tgaggtgtct 250
 LeuPheLeuL euLeuLeuTr pLeuThr
 ttgcacatca gtgaaaactc ctgccacctc tgctcagcaa gaaatataat 300
 taaaattcaa aatagatcaa caattttggc tctactcaaa gacagtgggt 350
 ttgattttga ttacatgagt gcattttctgt tttattttcca atttcagATA 400
 AspT
 CCACCGGAGA AATTGTGTG ACACAGTCTC CAGCCACCGT GTCTTTGTCT 450
 hrThrGlyGl uIleValLeu ThrGlnSerP roAlaThrLe uSerLeuSer
 CCAGGGGAAA GAGCCACCGT CTOCTGCAGG GCGAGTCAGG GTGTTAGCAG 500
 ProGlyGluA rgAlaThrLe uSerCysArg AlaSerGlnG lyValSerSe
 CTACTTAGCC TGGTACCAGC AGAAACCTGG CCAGGCTCC AGGCTCCTCA 550
 rTyrLeuAla TrpTyrGlnG lnLysProGl yGlnAlaPro ArgLeuLeuI
 TCTATGATGC ATCCAACAGG GCGACTGGCA TCCAGCCAG GTTCAGTGGC 600
 leTyrAspAl aSerAsnArg AlaThrGlyI leProAlaAr gPheSerGly
 AGTGGGCCTG GGACAGACTT CACTCTCACC ATCAGCAGCC TAGAGCCTGA 650
 SerGlyProG lyThrAspPh eThrLeuThr IleSerSerL euGluProGl
 AGATTTTGCA GTTTATTACT GTCAGCAGCG TAGCAACTGG CATCCACAG 700
 uAspPheAla ValTyrTyrC ysGlnGlnAr gSerAsnTrp His
 TGATTCCACA TGAACAAAA ACCCAACAA GACCATCAGT GTTTACTAGA 750
 TTATTATACC AGCTGCTTCC TTTACAGACA GCTAGTGGGG TGGCCACTCA 800
 GTGTTAGCAT CTCAGCTCTA TTTGCCATT TTGGAGTTCA AGTTGTCAAG 850
 TCCAAAATTA CTTATGTTAG TCCATTGCAT CATACCATT CAGTGTGGCT 900

FIGURE 42

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CCGCCCCAGC TGCTTTGCAT GTCCCTCCCA GCGGCCCTGC AGTCCAGAGC 50
 CCATATCAAT GCGTGGGTCA GAGCTCTGGA GAAGAGCTGC TCAGTTAGGA 100
 ACCCCAGAGG GAACCATGGA ACCCCAGCG CAGCTTCTCT TCCTCCTGCT 150
 MetG1 uThrProAla GlnLeuLeuP heLeuLeuLe
 ACTCTGGCTC CCAGgtgagg ggaacatggg atggttttgc atgtcagtga 200
 uLeuTrpLeu Pro
 aaaccctctc aagtcctgtt acctggcaac tctgctcagt caatacaata 250
 attaaagctc aatataaagc aataattctg gctcttctgg gaagacaatg 300
 ggtttgattt agattacatg ggtgactttt ctgttttatt tccaatctca 350
 gATACACCG GAGAAATTGT GTTGACCGAG TCTCCAGGCA CCGTGTCTTT 400
 AspThrThrG lyGluIleVa lLeuThrGln SerProGlyT hrLeuSerLe
 GTCTCCAGGG GAAAGAGCCA CCTCTCTCTG CAGGGCCAGT CAGAGTGITA 450
 uSerProGly GluArgAlaT hrLeuSerCy sArgAlaSer GlnSerValS
 GCAGCAGCTA CTTAGCCTGG TACCAGCAGA AACCTGGCCA GGCTCCAGG 500
 erSerSerTy rLeuAlaTrp TyrGlnGlnL ysProGlyGl nAlaProArg
 CTCTCATCT ATGGTGCATC CAGCAGGGCC ACTGGCATOC CAGACAGGTT 550
 LeuLeuIleT yrGlyAlaSe rSerArgAla ThrGlyIleP roAspArgPh
 CAGTGGCAGT GGGTCTGGGA CAGACTTCAC TCTCACCATC AGCAGACTGG 600
 eSerGlySer GlySerGlyT hrAspPheTh rLeuThrIle SerArgLeuG
 AGCCTGAAGA TTTTGCAGTG TATTACTGTC AGCAGTATGG TAGCTCAOCT 650
 luProGluAs pPheAlaVal TyrTyrCvsG lnGlnTyrGl ySerSerPro
 CCACAGTGA TTCAGCTTGA AACAAAAACG TCTGCAAGAC CTTCAATTGTT 700
 TACTAGATT TACCAGCTGC TTCTTTTACA GATAGCTGCT GCAATGACAA 750
 CTCATTTEAG CATCTCTCTC TGCTTGGGCA TTTTGGGGAT CTTAAAAAAG 800
 TAATCCCTTG ATATATTTTT GACTCTGATT CCTGCATTTT TOCTCAGACC 850
 AAGATGGACA GCCAGGTTTA AGCACAGTTT CACAGTAATG GCGACTGGAT 900

FIGURE 43

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AAACACATTC TCTGCAGACA AATTTGAGCT ACCTTGATCT TAOCTGGACA 50
 GGTGGGGACA CTGAGCTGGT GCTGAGTTAC TCAGATGGGC CAGCTCTGCA 100
 GCTGTGCCCA GCGTGCCCA TCCCTGCTC ATTTGCATGT TCCCAGAGCA 150
 CAAOCTCCTG CCGTGAAGOC TTATTAATAG GCTGGTCAGA CTTTGTGCAG 200
 GAATCAGACC CAGTCAGGAC ACAGCATGGA CATGAGGGTC CTGCTCAGC 250
 MetAs pMetArgVal LeuAlaGlnL
 TOCTGGGGCT CCGTCTGCTC TGTTCCTCAG gtaaggatgg agaacactag 300
 euLeuGlyLe uLeuLeuLeu CysPhePro
 cagtttactc agcccagggg gctcagtact gctttactat tcagggaaat 350
 tctcttaciaa catgattaat tgtgtggaca tttgttttta tgtttccaat 400
 ctcagGTGOC AGATGTGACA TOCAGATGAC CCAGTCTCCA TOCTCACTGT 450
 GlyAla ArgCysAspI leGlnMetTh rGlnSerPro SerSerLeuS
 CTGCATCTGT AGGAGACAGA GTCAACATCA CTTGTGGGGC GAGTCAGGGT 500
 erAlaSerVa lGlyAspArg ValThrIleT hrCysArgAl aSerGlnGly
 ATTACGAGCT GGTTAGCCTG GTATCAGCAG AAACAGAGA AAGCCCTTAA 550
 IleSerSerT rpLeuAlaTr pTyrGlnGln LysProGluL ysAlaProLy
 GTCCCTGATC TATGCTGCAT CCAGTTTGCA AAGTGGGGTC CCATCAAGGT 600
 sSerLeuIle TyrAlaAlaS erSerLeuGl nSerGlyVal ProSerArgP
 TCAGCGGCAG TGGATCTGGG ACAGATTICA CTCTCAACAT CAGCAGCCTG 650
 heSerGlySe rGlySerGly ThrAspPheT hrLeuThrIl eSerSerLeu
 CAGCCTGAAG ATTTTGCAAC TTATTACTGC CAACAGTATA ATAGTTACCC 700
 GlnProGluA spPheAlaTh rTyrTyrCys GlnGlnTyrA snSerTyrPr
 AOCACAGTG TTACACACCC AAACATAAAC CCCCAGGGAA GCAGATGTGT 750
 GAGGCTGGGC TGCCCCAGCT GCTTCTCTG ATGCTCTCAT CAGCTGAGAG 800
 TGTTCCTCAG ATGCAGCCAC ACTCTGATGG TGTGGTAGA TGGGGAC 847

FIGURE 44

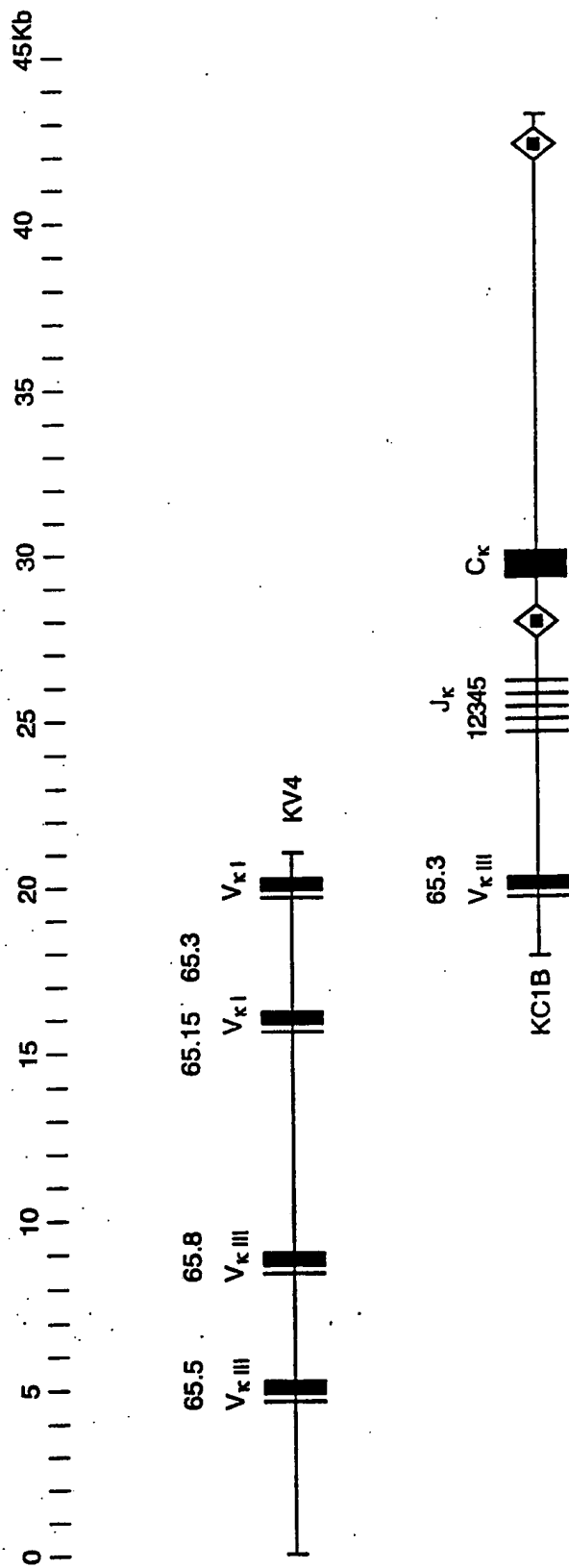


FIG. 45

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/10983

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 15/00

US CL :800/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; heavy chain transgene
light chain transgene**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A, 90/04036 (Bruggemann <u>et al</u>) 19 April 1990, see entire article.	1-11, 12-26, 27, 28, 29-32, 33
Y	Nature, Volume 342, issued 23 November 1989, Zijlstra <u>et al.</u> , "Germline transmission of a disrupted B2-microglobulin gene produced by homologous recombination in embryonic stem cells", pages 435-438, see entire article.	35-39, 43-53
Y	Nature, Volume 350, issued 04 April 1991, Kitamura <u>et al.</u> , "A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin u chain gene", pages 423-426, see entire article.	35-39, 54-58, 61-65

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

03 MARCH 1993

Date of mailing of the international search report

07 APR 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/10983

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/10983

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chemical Reviews, Volume 90, No. 4, issued June 1990, Uhlmann <u>et al.</u> , "Antisense Oligonucleotides: A new Therapeutic principle", pages 544-584, see entire article.	34, 40-42, 59, 60